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ANNUAL REPORT

MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM -
Division of Clinical and Behavioral Research,
Division of Biological and Biochemical Research, and
Division of Special Mental Health Research

United States

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1978 - September 30, 1979

VOLUME I

Summary Statements

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ANNUAL REPORT
# of the
DIRECTOR, MENTAL HEALTH INTRAMURAL RESEARCH PROGRAM
NATIONAL INSTITUTE OF MENTAL HEALTH
October 1, 1978 - September 30, 1979

John C. Eberhart, Ph.D.

This report is being written six weeks after the close of Fiscal 1979, and is thus late. It is embarrassing to be late with an annual report, especially just after one has been dubbed a "senior executive" by the Civil Service Reform Act (CSRA). One would assume that senior executives, at least good ones, would prepare annual reports promptly, as they do other minor chores and not drag the job out whatever the excuse. In my case the excuse seems to be a heavy investment of time throughout the year, and especially during the summer and early fall, with the new creation of the CSRA--the Senior Executive Service. The language of the CSRA converts virtually all of our senior scientists into "senior executives," and since the SES sets up a new system of cash bonuses for its members, and by implication defines their motivations and incentives in its own idiosyncratic way, it is a serious matter.

I commented at some length about the proposed SES in this space last year, prior to final passage of the bill. On the basis of a year's experience with the implementation of the Act, I want to tell what has happened and discuss its present and future impact. The bulk of this report will deal with that.

Before going on to these matters I want to direct the reader's attention to the summaries of this program's scientific research prepared by the Laboratory and Branch Chiefs. These annual summaries provide a review of the year's efforts and achievements. The basic science laboratories, several of which are also studying clinical problems, and the clinical research branches, most of which are carrying on some basic research too, are under splendid, proved leadership and have been very busy and productive. In a number of areas new and surprising findings of great potential significance have been reported this year. The outlook for the next two or three years is a bright one, especially if we can continue to provide the resources needed. Perhaps the central issue here is whether our personnel ceiling is permitted to match the level of our budgeted positions. In FY 1979 our budgeted positions, as provided in the HEW appropriations, numbered 428. Our personnel ceiling was, however, limited to 400. The other 28 positions were part of the 40 appropriated in 1979 to establish a research program in geriatric psychiatry but which we were prevented from filling by first a freeze and then a lowered ceiling. Until the 28 or a major portion of them can be made available to us the geriatric psychiatry research will have to develop quite slowly, and the inpatient clinical phase cannot be started.

Despite the advent of the SES, and its junior counterpart, the Merit Pay System, the laboratory research programs have not, so far as I can tell, been adversely affected. So far, at any rate. Of course the newly prescribed form of performance appraisal was not activated until after July 1 for the SES, and in November for the Merit Pay System members and no one in either system has received a bonus or had one withheld. The real test will be what happens after July 1, 1980, when performance appraisals are to be made and decisions on pay are reached. During the year we did what we could to acquaint members of the staff with the coming changes, but it may be that in these preliminary stages, the changes lack a certain reality. Civil servants have had to learn not to cry until they are really bitten. It could certainly not be said that the new laws lacked adverse impact because they were popular. Aside from the installation of paid parking, which began at NIH and elsewhere on November 1, 1979, I cannot recall any change in working conditions that has been less popular than the SES and the Merit Pay System.

My participation in this set of personnel and management issues involved several kinds of activities. The first was as a delegate from NIMH and ADAMHA (and NIH too, it turned out) to the large Ocean City conference organized by the Civil Service Commission in October 1978, right after passage of the CSRA. I found out there that the major issue, for me and for intramural research generally, was how senior scientists--generally at the lab chief level--could work within the philosophy and rules of the SES. The system seemed clearly designed for general administrators, and certainly not for scientists. How could the performance of scientists be properly evaluated on a 12-month basis? What justification could there be for giving a bonus to one good scientist, and withholding bonuses from other scientists almost or entirely indistinguishable in quality? I used my time at the conference to question CSC staff about the adaptability of the SES system to a scientific work force, and at the least I and others succeeded in alerting some of them to the fact that there was an issue.

After the Ocean City conference the HEW Department set up a number of task forces to deal with the many issues raised by the CSRA. One dealt with the SES, and I have served on it throughout this period. The Assistant Secretary for Personnel, Thomas S. McFee, set up a management advisory group composed of representatives from many agencies, and I served on that too for the two or three meetings that were held. The NIH, after discussion by the Board of Scientific Directors, set up a committee on performance appraisal of scientists, and I served on that. Mrs. Hazel Rea represented NIMH and the IRP on a PHS Committee on the SES. These committees and task forces over the course of the year consumed several weeks of time, as did the later briefings, orientations, and interviews with Scientific Directors, Lab Chiefs, and SES members in ADAMHA and the NIH.

Among the issues debated in these groups were:

- What functions define an "executive" and thus determine qualifications for SES membership?
- Can scientists fit into a system designed for "executives"?
- How rapidly should the SES be organized and installed?
- At what level or levels in the Department is the SES to be administered?

- How many Performance Review Boards will be needed and in what organizational segments will they be located?
- Can agency heads appoint their own PRB's?
- How will authority for making key decisions about the SES be distributed among Office of Personnel Management, the Secretary's Office, the heads of Principal Operating Components (POC's), and the agencies?
- What form of performance appraisal will be used? One prescribed by the Department, or several as needed by the diverse POC's and agencies?
- What will be the functions and authorities of the Executive Resources Boards, and at what levels will they be located?
- How should a subset of the SES, designed for intramural scientists at NIH/NIMH, be designed?
- What size bonuses should be permitted? What should be the criteria for bonuses?
- How should salary ranks (grades) be determined? Should an SES member be able to get both a bonus and a raise in salary in the same year?
- What should a performance appraisal system for scientists consist of?

This list could be extended almost indefinitely. Though the language of the CSRA was unusually detailed and specific, much had to be thought through and decided before the new statutory provisions could be satisfactorily adapted to the realities of this Department.

In one way we were favored in this exercise. The OPM had urged, at Ocean City and on other occasions, that there be maximum delegation of authority under the CSRA, including authority for the administration of the SES. In the advisory committees we urged that authority be delegated by the Department to the POCs, with the possibility for redelegation, so that, for example, the NIH with its unique intramural research programs would be able to develop its own performance appraisal system, appoint its own Performance Review Boards, and administer the bonus system with its own criteria. Since the Department was later to agree to the designation of a "Scientific Service" as a subset of the SES, and since all intramural SES scientists (including those from ADAMHA) would be included in it, that was an important issue. Subsequently the NIH personnel staff, with suggestions from the Scientific Directors advisory committee, worked out a system of "performance assessment" for the Senior Scientific Service, and we have been operating under it since July, 1979.

In this connection I want to pay tribute to the outstanding work of the two persons who contributed most to design of the SES and its appraisal system. They are Ms. Anabel L. Holliday, Assistant Director for Policy of the NIH Division of Personnel Management, and Ms. Melissa Howard, a member of her staff. In a situation where the SES was viewed with foreboding by almost all scientists, and where the implications of the system were very discouraging, these two ingenious, creative, and buoyant experts proceeded to develop the structure of a Scientific Service and to design an appraisal system which could be accepted by scientists. Their work was essential to NIH participation in the SES. Their perserverance, ingenuity, and resourcefulness were exceptional. As a result of all this activity a good deal has been achieved during the year. I would like to summarize what has been done, where we are now, and what the implications seem to be for intramural research at NIH/NIMH.

1. The NIMH Intramural Program, on the basis of criteria developed by OPM, asked for 38 SES slots. This number would cover all our 210(g)'s, GS-16-18's senior PHS Commissioned Officers who would merit a supergrade in Civil Service; unfilled supergrade positions; and any GS-15's who were believed qualified for supergrades. We were given 21, only a little better than half the number we believed we needed. Commissioned Officers were excluded by OPM policy, as were GS-15's. The 21 slots took care of our immediate needs, except for two Commissioned Lab Chiefs, both former GS-17's, who wished to be transferred from the Commissioned Corps to the SES. (In the 7 months since then we have been unable to get Departmental and OPM approval for the straightforward transfer of senior research scientists from the Corps to SES.) One of our 210(g)'s declined the invitation to join the SES, and the SES slot reserved for him was claimed by the NIMH. We now have 20; 17 are filled, one is reserved for a Director of the Division of Biological and Biochemical Research, and the other two are reserved for chiefs of the Laboratory of Psychology and Psychopathology, and the Geriatric Psychiatry Branch.

The matter of numbers of slots would not be important if additional slots were readily available. They are not. The Department, with a total of about 780 SES slots, has about 40 in reserve. We have been told that we cannot ask for additional ones as long as we have unused ones, and since recruiting for one of these positions is often a long proposition, we will either be prudent, and maintain a vacancy for the end of the long recruiting process, or be imprudent, use up the vacant slot for an existing need and run the risk of not getting another when the recruitment finally comes through. Though it was my impression during the campaign to pass the CSRA, and at the Ocean City Conference after passage, that there would be plenty of SES slots for legitimate supergrade appointments in the Government as a whole, subsequent events have cast great doubt on this. Legitimate requests for slots have been turned down, and I am pessimistic about the supply situation. More about this later.

2. A Senior Scientific Service (SSS) has been set up by the NIH, with approval by the Office of the Secretary, as a subset of the SES. The SSS includes all SES members in NIH and ADAMHA whose duties are in direct research or research administration, rather than in extramural programs.

The NIMH-IRP SES members are in the SSS. The provisions of the CSRA dealing with the SES all apply to SSS members, but there are differences in the specifics of the performance appraisal system and in makeup of the relevant Performance Review Board. For the SSS the NIH Board of Scientific Directors will be the PRB. It will be supplemented by as many other SSS members as needed; it will in all probability work as a series of panels, each of which will review 20-25 appraisals. The appraisal system designed for the SSS will be used also for those members of the Merit Pay System who are in intramural research. The SSS, by its title, seems to make it clear that scientists are legitimate members of the SES and that they need not regard themselves as nor attempt to become "executives," if that term means something besides being an effective leader of a research group.

3. Much of the policy-setting authority for the SES resides in a series of Executive Resources Boards. There will be one such board for each POC, and a senior board in the Office of the Secretary. A board at the PHS level will govern matters in the NIH, ADAMHA, and other health agencies. Chairman of that board is Mr. Charles Miller, Deputy Assistant Secretary for Health Operations; the ADAMHA representative is Mr. Robert Trachtenberg, Deputy Administrator, and the NIH member is Dr. Thomas Malone, Deputy Director. The administrative emphasis in the membership of such boards is apparently intentional. The relative absence of substantive expertise, especially in research which is the principal focus of more than half of the PHS SES members, is notable. If good consultation can be developed between science program managers and members of this board it may work out reasonably well.

4. As mentioned above, the Department approved the delegation of authority to devise performance appraisal systems and to appoint Performance Review Boards to the principal operating components of the Department, including the PHS, and the latter redelegated these authorities to the agencies. These decisions permitted the NIH, with its distinctive research mission and large number of SES slots (NIH has 210 SES slots; together with the 20 in the NIMH-IRP, there are 230 on the NIH campus, out of 437 allocated to the PHS) to attack and solve many of its own problems.

5. The matter of "performance appraisal" for scientists in the SES is crucial, since the results of such appraisal will determine who gets bonuses and how much, and who gets or loses salary, and how much. In addition, of course, such a formal appraisal process can have--will probably have--a psychological impact, for good or ill, on those subject to it, and on the relationship between appraisor and appraisee. Both kinds of impacts--on money and on self-esteem--worry me and others. Science is different from most other occupations in that (1) The taskmaster for the scientist is not or should not be, his boss. It is nature whose secrets he is trying to discover. (2) The time scale for the scientist's achievements often is years, even decades, rather than months, although there often are research tasks of lesser importance that can be carried out in shorter periods. The problem facing those of us who worked on the performance appraisal system was to devise something that dealt with the heart of the scientist's task--discovering new knowledge--rather than with more superficial and less relevant matters such as the way he went about it. We did not want to require the use of artifactual quantification--numbers that would yield a score, but which would not truly

represent the appraiser's judgement. We did want to concentrate on the best evidence we could get as to his success in research, and given the nature of science we knew that often that would be the subjective judgement of an informed peer, or supervisor.

Most of the work of devising a performance system was done by Melissa Howard, in consultation with a number of scientists. The system she devised was tried out by us and by the NIAMDD members of the SSS and was then somewhat revised. It does not commit the sins mentioned above, and it does concentrat on the primary functions of the scientist. It requires the rater to review and evaluate indicators of success or lack of it in carrying out the major responsibilities of the position. He then chooses one of the following categories to represent his judgement of the scientist: outstanding, highly successful, fully successful, minimally satisfactory, unsatisfactory. Whether such judgements will permit us to distinguish between those who should get bonuses and those who should not remains to be seen. I have yet to see any measurement system which provides a meaningful dividing line at the 50 percent point in the distribution, as the CSRA apparently requires us to do with the giving and withholding of bonuses in the SES.

6. Salary levels in the SES are 6 in number and range from ES-1 at $44,756 to ES-6 at $52,800. The salary figures are important because this portion of the total compensation continues from year to year, and it is the only form of compensation that is involved in computing annuities. Bonuses and Executive Ranks ("Meritorious Executive," "Distinguished Executive") are jump sum one-time payments, not calculated into the retirement formula. The range of salary levels as will be seen from the figures above, is too small to be meaningful. Because of the compression of supergrade salaries up against the Congressionally imposed ceiling, more than 90 percent of those converted into the SES were at one salary level, $50,112 (ES-4). The SES Task Force has wrestled with the issue of criteria for determination of salary levels, with some members feeling that since bonuses are to be based on "performance," salary should be based on something else. But what is there other than performance? Responsibility? Difficulty? No neat resolution has been achieved. I have argued, hardly at all cynically, that the wise SES member will regard salary, bonus (if any), and Executive Rank (if any) as dollars, that the total compensation is important, but that the salary dollars are more important than the others, for the reasons given above. For these reasons salaries, as well as bonuses, should be available for flexible use to reward good staff, attract superior outsiders to the staff, and outbid competitors who are trying to hire our good people away. This isn't neat but I think it is realistic.

7. As mentioned above we proceeded during the year to acquaint our senior scientists with the SES and its implications. Several NIMH scientists provided advice in working out the appraisal system. In July all SES members in NIMH and NIAMDD intramural staffs worked out what is coming to be called "work plans," in meetings with the respective Scientific Directors. Although this was a preliminary run-through to try out the system, it appeared in October when the official work plans were scheduled to be prepared that the July exercise had pretty well accomplished the October purpose. On November all NIMH-IRP work plans were submitted to ADAMHA at the request of Mr. Trachtenberg, Deputy Administrator.

8. There are several tasks remaining to complete the first year of actual operations under the SES. They include:

(a) Preparing nominations of any staff to be recommended for the ranks of Meritorious Executive or Distinguished Executive. The former carries a stipend of $10,000 and may be given to not more than 5 percent of career SES members (that means 39 in HEW). The latter carries a stipend of $20,000 and the quota is 1 percent (7 in HEW). These nominations have to be in to OPM by April 15, 1980, three or four months before the performance appraisals are completed for the first year. These executive ranks are supposed to lay more emphasis on career contributions than on the prior 12 months performance.

(b) Beginning about July 1980, each SES member will have his performance appraised by his supervisor. There will need to be a meaningful and understanding kind of interaction between the two for the process to work well. I suspect that some of us will find that we have much to learn before we can be very good at either appraising or being appraised. The results of the appraisal sessions will then have to be reviewed by the Performance Review Board, and finally by the Institute Director and Agency Administrator. At the end of this process the recommendations made by supervisors for bonuses and salary adjustments will go into effect if approved by the PRB and the deciding official (Institute or Agency head, to be decided). It is intended by the Department that bonuses will be paid in September, before the close of the fiscal year.

(c) The number of bonuses that can be given remains to be determined. The law limits bonuses to career members of the SES, and provides further that the number of bonuses given may not exceed 50 percent of the number of SES positions. Since some SES positions are occupied by non-career members (who are ineligible for bonuses), and since other SES positions are likely to be vacant when decisions on bonuses are made, it is clear that more than 50 percent of career SES members may be eligible. In early November a calculation showed that 60 percent of PHS career SES officers would have been eligible. For the Department as a whole the figure was 71.5 percent. These figures may well change by next July. Furthermore, it is impossible to predict (1) how many affirmative bonus decisions will come out of the lengthy appraisal/review process, or (2) whether the budget-cutting and anti-inflation forces will pressure the Administration into placing an arbitrary limit on this particular expenditure.

(d) The size of bonuses is a question not answered by the law. A bonus may not exceed 20 percent of salary, but it may be anything under that. It has been argued in the Task Force that there should be a minimum bonus figure, and that it should be 3 percent or 5 percent of salary, but no decision has been made to date. It seems to be assumed that bonus size will follow adjectival rating in some way--that those rated "outstanding" will receive more than those designated "fully successful." In contrast to the merit pay system to be discussed below, the availability of funds to pay SES bonuses does not seem to be a problem. The law places no limit on funds used for this purpose. To give some idea of the magnitudes involved, it would take only a little over $5 million to pay the maximum 20 percent bonus to all the career SES members in the Department, (an unlikely event), and half that to pay bonuses averaging 10 percent to the same group. The funds can come from any appropriate budget item, and should not be a problem for most agencies.

9. The Merit Pay System. Though these comments are primarily concerned with the Senior Executive Service, it is necessary to record some observations about the other major innovation in the CSRA--the Merit Pay System (MPS). This applies the same "pay for performance" philosophy embodied in the SES to the large group of GS-13-15 supervisors in Government. Scientists are included like everyone else if they exercise supervision. Unfortunately, the design of the system is such as to raise serious questions about its long-term workability.

As to numbers, let me say that when designations for the MPS had to be made in June, 1979, the IRP had 51 employees in GS-13-15, of whom 38 qualified as supervisors, and thus for membership in the MPS. This is twice the size of our SES group. The 38 will be affected by the following provisions: Merit Pay members will no longer receive either regular in-grade raises or quality in-grades. Furthermore, they will receive only half the comparability pay raise (the "cost of living" raise) given other Government employees. The dollars which would have gone into these two forms of compensation will be set aside as a pool from which the best performing members will receive an unspecified amount of "merit pay" at the end of each year. The pay pool is inelastic, and it is small. It will probably not exceed 4 percent of the salaries of merit pay members. It is intended that merit pay be distributed unequally, so that the best performers get more and the worst performers get none. At least there is no requirement, as there is in the SES, that limits the recipients of merit pay to an arbitrary portion of the group, such as half. Merit pay members will undergo a performance appraisal, as do SES members, on the basis of which merit pay will be awarded. Merit pay constitutes an increase in salary, not a bonus. It therefore affects retirement annuity. It may not increase total pay beyond the top salary for the grade. Clearly some Merit Pay members, perhaps many, will receive no merit pay, and they will thus lose ground salary-wise as compared with peers not in the Merit Pay System. It is this last feature which has caused the greatest concern among merit pay members and managers of their enterprises.

There is no problem about either denying a pay increase to a subpar staff member or increasing the pay of a clearly superior employee. But large numbers of satisfactory or "fully satisfactory" supervisors will have to be denied any merit pay if the highly successful and outstanding workers are given merit pay in an amount which is a significant reward. It is impossible in my view, to justify denying to satisfactory employees the routine compensation increases which go to all their non-supervising peers. The argument could be made that a worker should not choose to be a supervisor if he does not wish to run that risk, but since it is almost impossible (or at least, very difficult in most occupations) to do high level work in a large bureaucracy without supervising someone, the option of staying out rather disappears. The possibility of widespread employee dissatisfaction, and very considerable bitterness, after merit pay is first awarded seems real. In the long run if this system is to work the CSRA should be amended to remove limits on the size of the merit pay pool. Merely satisfactory employees should be able to do as well under Merit Pay as their peers do outside of it.

10. An experimental personnel system for NIH intramural scientific staff. One of the useful provisions of the CSRA is the authority given to the Office of Personnel Management (OPM) to approve up to 10 experimental or demonstration personnel programs in as many Government agencies, each program limited to not more than 5,000 employees and a duration of 5 years. Since the provisions of the SES and the Merit Pay System are badly designed for scientists (for reasons discussed above and below) and since the job classification approach of even the traditional Civil Service works for scientists only because of some tortuous interpretations and adaptations, I and others have urged the NIH to take advantage of the new law and to outline a plan which can be submitted to OPM for approval. NIH has agreed to do so, and has opted for a plan which deals only with compensation rather than with such other matters as the position classification system, job titles, sabbaticals, consulting and other outside work privileges, and the like. On tactical grounds this choice can be supported, because proposed experimental programs have to go over a good many hurdles in the Department, OPM and the Congress before approval can finally be given. A comprehensive or ideal plan, designed to remedy all the supposed ills and frustrations of the intramural scientific staff, would probably have hard going.

Unfortunately, at the first go-round, the NIH request was combined by the Department with a request for another plan that would cover all personnel in the Center for Disease Control (CDC). The resulting document was poorly thought through and badly written, as I pointed out in a memo to the Director, Division of Personnel Management, NIH. I said, among other things, "The justification for an NIH demonstration project in my view was that (a) the salaries of scientists are too low, (b) the bonuses mandated by the CSRA for the SES are exactly the wrong way to motivate and reward scientists, and in the long run will corrupt the system, (c) the "merit pay" system of the CSRA is badly designed and unsatisfactory for all staff that come under it, including the scientists, (d) annual performance appraisals are too frequent to be meaningful for evaluating the work of scientists, and (e) the traditional CSC job classification system is ill-suited for scientists and should be replaced by a university-type "rank-in-man" system."

I pointed out that the draft plan proposed almost nothing that would be a solution to any of these needs, and that the heavy emphasis on job classification, short term performance appraisals, bonuses, and a simplistic theory of employee motivation were all going in the wrong direction. It further seemed to me that the proposed plan contemplated such heavy and continuous involvement in it by NIH/NIMH scientists that it would seriously interfere with their work. In my view any proposed experimental personnel plan for a scientific staff should first of all be simple and direct and not constitute an undue burden on the scientists, because if it cannot satisfy that need then it won't take care of other problems. The object of all administration, including personnel, in a scientific program is to provide for the needs of scientists in an unobtrusive way, thus to free their time and energies for the things only they can do--scientific investigation.

On the basis of these and other comments the Department agreed that combining a plan for the NIH intramural staff with one for all of CDC was probably not a good idea, and that the NIH should submit its own.

To conclude this recital of issues and events I want to make some summarizing comments about the Senior Executive Service and the whole new scheme of pay for performance. First, some gains and some losses as I see them.

A. Gains from the SES

1. Pay. Some senior staff will get more money, which most of them will deserve. Through use of the "bonus" device the pay cap established by the President and Congress has been punctured and that is a distinct gain. It is unfortunate that all deserving SES members will not be able to share in it.

2. Leave. SES members can now accumulate annual leave without limit, such leave being paid for in a lump sum upon retirement. This change seems to be welcome. The other side may be that some vacations will be foregone that should have been taken for the sake of rest, relaxation, and effectiveness on the job. We may never know.

3. Possible increased efficiency in the Civil Service. This is one of the hopes that led to the CSRA. (The other is increased responsiveness, discussed below.) The theory is that with more careful monitoring and evaluating by supervisors, and with the promise of bonus dollars as a reward for good work, many senior bureaucrats will exceed their prior levels of effectiveness. The argument is that this should produce an improvement in performance of the Federal system. One hopes that this will work. Since I do not believe that senior people work primarily for extra money, or for the approval of the supervisor, I am not sanguine about the outcome.

4. Greater responsiveness of the bureaucracy to administration initiatives. I think the extent of bureaucratic non-responsiveness has been exaggerated; I also think that some of what has appeared to be lack of response has really been in the national interest. Inaction is often better than bad action, and the number of foolish initiatives that can be thought up by fresh (and naive) political appointees is large. Nevertheless, a new administration has the responsibility to initiate new programs, and the right to expect that permanent civil servants will work vigorously in the pursuit thereof. Some of this will probably happen as a result of "pay for performance." Whether it is in the national interest to diminish the arena for independent judgement by senior civil servants, and to entice them into compliance with other priorities by the use of bonuses, is another matter. How it looks probably depends on where you sit.

Here are the costs, or losses; as I see them:

1. Loss of 210(g) appointments. The upgraded 210(g) appointments, though limited in number, were a boon in intramural research. They covered the supergrade range in salary, pay could be set administratively, no further promotion actions were ever necessary, and recruitment procedures were simple. Such appointments are now gone--they are a distinct loss.

2. Loss of unrestricted supergrades. For a number of years, there has existed an unlimited pool of supergrade positions for natural scientists and those in the medical and engineering sciences. Outside these scientific disciplines supergrades were restricted in number to a quota approved by Congress. Restricted supergrades were almost never available; unrestricted supergrades were always available to a properly qualified scientist. The promotion of a GS-15 scientist to GS-16 was never held up in the search for a slot. There was no table of organization which restricted the number of positions at any grade. Therefore, the scientist could go as fast and as far as his ability and achievements would carry him, unhindered by artificial barriers. This was a very important asset, and an essential feature of a good career system for scientists.

It is now gone. A few supergrades remain for non-supervisors (517 for the whole Government), but the unrestricted supergrades for scientists have been abolished. The only way to promote a GS-15 scientist now is to find a way to get him or her into the SES, and that has turned out to be much more difficult than anyone I know anticipated.

3. Loss of ability to promote above GS-15 on the basis of gradual assumption of duties. The OPM has so far refused to permit entry into the SES on the basis of a "gradual assumption of duties" promotion. The issue is sufficiently important that I prepared a statement on it for NIH and ADAMHA, and it follows as Appendix A. OPM currently insists that a full nationwide recruitment effort precede entry into SES, even if there is no vacancy to recruit for. Typically, a scientist in the NIH/NIMH intramural program is promoted when in the judgement of the NIH Scientific Directors his research achievements merit it. He continues in the same position. No vacancy is involved. This is called "gradual assumption of duties" because in the old fashioned Civil Service way of regarding grade and pay, they are awarded for characteristics of the position ("duties") rather than for qualities and achievements of the person. The phrase might better be "gradual increase in scientific stature."

If the present policy persists we will have witnessed the virtual destruction of the upper end of the career ladder for scientists, as contrasted with scientist executives, at NIH/NIMH. Further details on this issue are in the memo in the Appendix.

4. Lengthening of the appraisal and reward chain. Prior to the SES the appraisal process was continuous and informal, and the rewards for scientists came from the inside (promotion, salary increases, and increased resources) and the outside (prizes, honors, the esteem of colleagues, etc.). The inside appraisals and rewards have been rather directly done--Lab Chief to Scientific Director to Board of Scientific Directors. Recommendations of the latter are generally accepted by the Deputy Director for Science. Under the SES the appraisal system is more formal, a good deal more work and time-consuming, and it and bonus actions will now need to go through a Performance Review Board, an Institute Director, and Agency Administrator, in addition to the former sequence. These changes are somewhat more than nuisance changes, but less serious than the tangible losses mentioned above.

5. The cost in time is heavy and will probably remain so. Probably a month of my time went into the SES this year, and an intramural Division Director recorded 50 hours on these matters. After the first year this will be reduced but not eliminated. One could justify such use of time if the process made important contributions to our mission, but if it does not the time needed is a serious drain.

6. Losses of mutual trust, self-esteem, and job satisfaction. Much could be said about the probable psychological distresses that will result from the new systems, but I shall just note them in passing. These comments are predictions, not observations, and they may turn out to be wrong.

I am not here concerned with employees whose work is unsatisfactory (though their welfare must continue to be a matter of concern), but rather with those who are judged "fully successful" according to the new terminology. Trust between people is important, and between close associates is necessary. Up to now we have depended on a high level of trust, though the trend in recent years in Government has been to increase inspections, monitoring, audits, layers of review, and other procedures to insure "accountability." This is so pernicious a trend that it merits a separate essay. Suffice it to say that the SES pushes us farther along the same road. There is an emphasis on supervision and monitoring, on prescribing goals for employees rather than encouraging them to work out their own, on tangible evidence of success and on formal and in many instances on quantitative appraisals of performance. All of these are, in theory, a part of the process of supervision, but when they are prescribed and spelled out by law they significantly reduce the possibility of mutual respect and a kind of unspoken trust between colleagues.

Studies of employee groups report that 80 percent or more customarily rate their performance in the upper half of the distribution. It is difficult for most of us to recognize or admit that we are below average in anything as important as work or professional skills. Yet the SES, by its use of bonuses, is going to force approximately half of this selected group of senior Government executives and scientists to recognize that they are in the lower half because they do not qualify for a bonus this year. Self-esteem is a somewhat delicate flower, but it is an important component of life satisfaction, job satisfaction, morale, etc. How many "fully successful" executives who do not qualify for bonuses will begin to do worse rather than better, and feel worse about their jobs, their agencies, and the Government? Can removing the illusions about quality of performance be helpful at all? Is it conceivably therapeutic or cost-effective to compel employees to "face reality" in this way? The CSRA says the answer to this question is yes. I think the answer is no.

7. Money incentives vs. other values. The boss, in Government employment as well as in the private sector, generally has enough clout to secure reasonable respect and compliance from his staff, even though he does not have tangible rewards which he can quickly bestow. Under the SES and the Merit Pay plan, however, he now has bonuses and extra pay to

use as tangible expressions of his approval or disapproval. I see two losses here. On the one hand some of the staff under these plans may reorder their priorities so that "please the boss" comes before "do a good job." What is done then depends on how the boss and his wishes are perceived. In research this could be serious. It could affect choice of problem, superficial rather than intensive attack, opting for number rather than quality in publications, and even, God forbid, research design and interpretation of data. The rewards for success in science are already prominent enough so that the possibility of corruption exists (and occasional examples come to light). Should one dangle sizeable cash bonuses as rewards for a 12 month effort in front of basic scientists? I think there is a certain danger.

The converse of this is also present. That is, the inference that any action in support of a boss or his ideas is simply bucking for a bonus. This degrading assumption will not add to the civility of discourse. To put these two together, they say that the larger and more prominent the rewards, the greater the tendency to work for the rewards rather than for the goals, and the greater the likelihood of being perceived as working for the rewards. Both are a kind of corruption and I think both are in some measure unavoidable under the new systems.

* * * * * *

I will close this discussion of the SES with a final comment about its central theme--pay for performance. That tactical position rests on what is at best too simple an account of how humans are motivated. People do indeed work for rewards, and for some the most effective reward may be money. Especially if they have very little of it. For many others money is not the most powerful incentive, especially if working for it conflicts with other values. If by statute money is made into the principal, or only goal for senior executives in Government, and if the conditions of work are reshaped to emphasize this (work planning, performance appraisal), then we had better have a group of executives who are content to work for money, or we're in trouble.

The Government, you see, is not just like private industry. (As a matter of fact, much of private industry is not like the conventional view of private industry). Industry can, in theory, arrange a tight hierarchy of supervision in a company from the chairman of the board on down, see to it that those positions are all staffed with experienced, competent company executives, and then use carrot and stick in whatever proportions they choose to drive or seduce employees into performance of the desired quality. Tight control characterizes such a system. With enough control, authority to hire and fire, and enough other rewards and punishments it doesn't matter, it may be argued, whether the employees like the system or not--one can still get production, or performance. But the Government is not like that, and it will not in the near future become like that.

Activities of Government get started because of political decisions, and not all fit everybody's taste. They are staffed and run by heterogeneous groups of Federal employees, excellent, good and indifferent. Few employee groups are bad. In charge are executives of varying degrees of expertise, some politically appointed and some career civil servants, but characteristically their tenures are short, and often their knowledge of program is even scantier. One might wish for a tightly controlled agency instantly responsive to orders from above, with staff rewarded and punished as surely and reliably as the sun rises, but the capacity for that is not there. The Civil Service Reform Act can't recreate all Federal agencies, but it can interfere with the middling successful way they have been conducting themselves over the years.

Some Federal agencies perform poorly and some perform extraordinarily well. The NIH, in my view, is one of the latter. Good agency performance comes from a clear and understood mission, good leadership, a competent and highly motivated staff, a reasonable appropriation, and minimal interference from distant management levels. The CSRA, in its pay for performance philosophy, treats all agencies, good, bad, or indifferent, alike. All are to be made to perform by concentrating on the rewards and punishments for staff, as if that could cure the bad effects of unclear or conflictful missions, inexperienced and changing leadership, and interference from management levels that have ample power and little knowledge. There may be agencies that could use the SES and Merit Pay principles to advantage. No one could criticize legislation which offered this option to agencies. But it is very bad public policy to require all Government agencies, successful and unsuccessful alike, to fall into line with a management philosophy that may help the weak, but will certainly not help, and may well damage, the strong.

Administrative Notes

The Board of Scientific Counselors: The review and evaluation of the Intramural Program by the Board of Scientific Counselors required three meetings this year rather than the customary two. On October 27-28, 1978, the Board visited the NIH Animal Center in Poolesville and reviewed the Laboratory of Brain Evolution and Behavior, then returned to Bethesda to review the Laboratory of Clinical Neuropharmacology. On March 29-30, 1979, the Board devoted the full two-day meeting to a review of the large Biological Psychiatry Branch. We then scheduled a special meeting for May 31 and June 1, 1979 for a review of the Laboratory of Socio-Environmental Studies. Dr. David Hamburg was chairman during the year. His service on the Board, and that of Drs. Robert Doty and Frederic Worden, all of whom finished their terms this year, has been very valuable to this program, and we are grateful for their efforts on our behalf.

As the fiscal year ended we appointed two new members: Dr. Masakazu Konishi, Professor of Biology, California Institute of Technology, and Dr. J. Allan Hobson, Professor of Psychiatry, Harvard Medical School.

Organization: In March we finally completed the transition of the Section on Technical Development to branch status, as the Research Services Branch. Dr. Theodore Colburn continues as Chief of the Branch, and also as Chief of the Section on Instrumentation and Computers. Dr. Jere Phillips was appointed Chief of the Section on Laboratory Animal Medicine and Care.

Foreign Work Assignments: During the year two staff scientists were on foreign work assignments. Dr. David E. Barrett of the Laboratory of Developmental Psychology spent the year at the Institute of Nutrition of Central America and Panama in Guatemala, collecting data for a study of the effects of chronic malnutrition on children's behavior and development. Dr. Russell E. Martenson, biochemist in the Laboratory of Cerebral Metabolism, spent the year in Lausanne, Switzerland, studying the biochemistry of the myelin sheath basic protein in the Laboratory of Neurochemistry, Universitaire Vaudois.

Honors and Awards: I am pleased to report the following honors and awards received by members of the Intramural staff in 1979:

JULIUS AXELROD, Ph.D. Laboratory of Clinical Science, DCBR	Elected Foreign Member of the Royal Society of London (England)--for exceptional scientific achievement.
MICHAEL J. BROWNSTEIN, M.D., Ph.D. Laboratory of Clinical Science, DCBR	Honored Alumnus Award, University of Chicago--for professional excellence.
ROBERT A. COHEN, M.D. Division of Clinical and Behavioral Research	Frieda-Fromm Reichman Award, American Academy of Psychoanalysis--for his important contributions toward a better understanding of schizophrenia and all its implications.
THEODORE R. COLBURN, Ph.D. Research Services Branch	Administrator's Award for Meritorious Achievement--for outstanding leadership in providing sustained high quality research support to IR investigators and for personal creativity in engineering design.
EDWARD V. EVARTS, M.D. Laboratory of Neurophysiology, DBBR	Degree of Docteur Honoris Causa, University of Brussels (Belgium)--for his valuable scientific contributions.
DANIEL E. GAITHER Laboratory of Neurophysiology, DBBR	Administrator's Award for Meritorious Achievement--for a high degree of initiative, conscientiousness, and care in the management of animal facilities, and for longstanding devotion to the NIMH and its mission.

ELLIOT S. GERSHON, M.D. (2 Awards) Biological Psychiatry Branch, DCBR	Anna-Monika Foundation Award--for research in endogenous depression.
	Commissioned Corps Commendation Medal in recognition of remarkable administrative and leadership ability and for developing new directions and new findings in biological genetic studies of psychiatric illness.
FREDERICK K. GOODWIN, M.D. Clinical Psychobiology Branch, DCBR	Centennial Lecturer, University of North Carolina School of Medicine--presented to a distinguished alumnus.
HAROLD A. GREENBERG, M.D. Division of Clinical and Behavioral Research	Administrator's Award for Meritorious Achievement--for sensitivity to research and patient care issues, by thoughtful adherence to high standards of medical ethics and numerous contributions to the maintenance of a therapeutic program equal in excellence to our research activities.
MELVIN L. KOHN, Ph.D. Laboratory of Socio-Environmental Studies, DCBR	Elected Fellow of the American Academy of Arts and Sciences.
IRWIN J. KOPIN, M.D. Laboratory of Clinical Science, DCBR	Plaque--The Dysautonomia Foundation --for distinguished research in dysautonomia.
MORTIMER MISHKIN, Ph.D. Laboratory of Neuropsychology, DCBR	Administrator's Award for Meritorious Achievement--for outstanding contributions to understanding the brain's structure and function through application of a multidisciplinary approach to the study of pre-frontal and inferotemporal cortex of the brain.
CANDACE B. PERT, Ph.D. Biological Psychiatry Branch, DCBR	Arthur S. Fleming Award--for identification of receptors on nerve endings which are specific for morphine-like substances and for the discovery of the first opioid compound in human plasma.

HAZEL W. REA Associate Director for Program Management, IRP	Administrator's Award for Meritorious Achievement--for outstanding contributions to the administration of Mental Health Intramural Research over the past 25 years.
DAVID SHAKOW, Ph.D. Division of Clinical and Behavioral Research	Distinguished Scientific Contribution Award, Maryland Psychological Association--for his distinguishe scientific contributions.
LOUIS SOKOLOFF, M.D. (2 Awards) Laboratory of Cerebral Metabolism, DBBR	Foster Elting Bennett Memorial Lecture, American Neurological Association--for distinguished contributions to the neurosciences. Grass Travelling Scientist, Society for Neuroscience--for deoxyglucose method for measuring brain function.

Staff Changes: Two members of our scientific staff retired during the year, and two others resigned to go to universities. Those retiring were Drs. Virgil Carlson and Albert Caron, long-time members of the Psychology Laboratory. Dr. Ronald Rieder, a newer member of the same Laboratory resigned to join the Psychiatry Department staff at Columbia University and Dr. Patricia Goldman, a senior member of the Neuropsychology Laboratory resigned to accept a Professorship in Anatomy at Yale. I would like to express our appreciation for the good work these scientists have done over the years for science and for the NIMH.

Another noteworthy change late in the year was the replacement of Ms. Bonnie Rubinstein by Mr. Michael Rosenthal as head of the Intramural satellite personnel office. Ms. Rubinstein transferred her activities to the ADAMHA personnel office. Because of the importance of personnel work in this program we are always concerned that the office be properly staffed; and we are therefore glad to welcome Mr. Rosenthal with his considerable experience in several NIH intramural research programs.

Special Event: My final note is to recollect the pleasant visit of Mrs. Rosalynn Carter to the Intramural Program on September 27, 1978. Though it lasted only an hour and a half it gave her and Secretary Califano, who accompanied her, a chance to see some of our research and to hear from some of our scientists. In addition to the two principal guests we also had Drs. Tom Bryant, Gerald Klerman, Dewitt Stetten, Herbert Pardes and Morton Lipsett. All of the guests and a small number of staff met for 15 minutes or so in 4N-226 for greetings and general explanations, and then walked around to visit Dr. Axelrod's laboratory, then to the day room on Ward 3-West where staff and patients had gathered, then to a pediatric ward on 9-D to visit a hyperactive child, and finally back to 4N-226 for final comments and a brief conference about the President's Commission on Mental Health.

A television crew from CBS followed the group most of the way. Dr. Axelrod described briefly some of his current research and commented on the importance of basic research for the development of clinical applications. In the day room Drs. William Bunney and Fred Goodwin talked about research on schizophrenia and on affective disorders and answered many questions from Mrs. Carter, the Secretary and others. In an adjoining room a TV tape of a depressed woman was played, giving in 3½ minutes a vivid picture of the pain and turmoil of the seriously depressed state. When the film concluded the patient who had been photographed was brought in and introduced to Mrs. Carter. She was remarkably improved and the whole incident was a very dramatic one. Just outside the TV room Dr. Chris Gillin demonstrated and explained the recording of EEG patterns from a sleeping normal volunteer.

On the 9th floor Dr. Judy Rapoport greeted Mrs. Carter and spent 10 minutes or so with her, the Secretary, and the TV crew in a bedroom with a hyperactive 3½ year-old boy. The presentation was well done and of obvious interest to the guests. Back in 4N-226 Dr. Thomas Wehr demonstrated the new activity monitor, one of which had been worn for 48 hours by a member of the Secretary's staff.

Comments afterward from Mrs. Carter and the others were complimentary about the visit and the presentations. I thought the latter were very expertly and simply done. And it seemed to all of us that the interest shown by the Secretary and the First Lady more than justified the considerable effort it took to plan and conduct such a visit.

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APPENDIX A

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health

MEMORANDUM

DATE: November 5, 1979

TO: Director, Division of Personnel Management, ADAMHA
Director, Division of Personnel Management, NIH

FROM: Director of Intramural Research, NIMH

SUBJECT: Unavailability of the Senior Executive Service to GS-15 Scientists Deserving of Promotion

The present policy of the Office of Personnel Management, I am informed, prohibits the promotion of a scientist from GS-15 into the SES by way of "gradual assumption of duties." The policy thus makes impossible the kind of promotion above GS-15 that has characterized almost all promotions of scientists at NIH/NIMH. If this policy is not changed the adverse impact on NIH's ability to provide scientific careers to world class scientists will be devastating.

In the attached statement I have outlined some aspects of the issue and some details on its probable consequences. The matter is serious, and its resolution is of very great importance to the continuation of first-rate intramural research at NIH. The options are clear: we either get the policy changed, or we (1) accept GS-15 as the defacto top salary for intramural scientists, or (2) be prepared to undertake a nationwide search to fill a nonexistent vacancy every time a good GS-15 scientist merits promotion. I do not see how we can live with either of these latter alternatives.

I urge that NIH and ADAMHA communicate these concerns to the Director of OPM with the request that promotion by "gradual assumption of duties" be recognized as a legitimate avenue for the entry of intramural scientists into the SES. Details of the issue are spelled out in the attached statement.

John C. Eberhart, Ph.D.

cc:
Director, NIMH
Acting Deputy Director, NIMH
Deputy Director for Science, NIH

Attachment

DISCUSSION PAPER

SUBJECT: Unavailability of the Senior Executive Service to GS-15 Scientists Deserving of Promotion

I. The Situation Pre-SES

1. Scientists in the NIH/NIMH intramural research programs have in the past been promoted to higher grades when review by their peers judges that their scientific achievements merit the higher grade. It is a variety of "rank-in-man" classification.

2. Before SES, the scientist, when being promoted above grade 15, would have been given a 210(g) appointment (if available), or a GS-16 from the unlimited number available to the medical and natural sciences and engineering.

3. While the number of scientists promoted above GS-15 has been small in comparison to the total, the ready availability of supergrades for highly meritorious scientists has been an important factor in retaining the best among the younger scientists. This is not solely a matter of money, for in many recent years senior GS-15's have been at or near the salary ceiling for supergrades. It is a matter of pride, because it represents a recognition by their peers of outstanding achievement.

4. Promotion of scientists to supergrades has never required that the scientist be promoted into a new position which has been classified in and of itself at a supergrade. The promotion recognizes "rank-in-man." Scientists also get selected to fill such positions as Section and Laboratory Chief, or Scientific Director, when they become vacant, and the added responsibility sometimes provides additional justification for a raise in grade. But it is a fact that the full range of grades has been available to scientists depending on their stature in world science and that it was not necessary that they assume responsibility for the work of a cohort of other scientists in order to be recognized by a supergrade. In characterizing such promotions the standard phrase is "gradual assumption of duties." A better phrase might be "gradual increase in scientific stature."

5. The typical "gradual assumption of duties" promotion from GS-15 to a supergrade is that of a Section Chief or Laboratory Chief, at GS-15, who is recommended for promotion to GS-16 (or 210(g)) because of recent outstanding research achievements. He does not shift positions. He is given a higher grade in his present position. No search is conducted for outside candidates because there is no vacancy to be filled. The promotion action consists of a reassessment of the candidate's scientific stature and a corresponding adjustment of his grade and salary.

II. The Situation Under SES

1. Such supergrade appointments as P.L. 313 and 210(g) are abolished and are thus no longer available.

2. Supergrade positions for those who are judged to be non-executive or non-supervisors are limited to 517 for the entire Government, and hence are by definition almost entirely unavailable. I understand that HEW has 24 of them, all usually full. The small number precludes any hope of their use for promotion of scientists above GS-15.

3. All other supergrade positions in the General Schedule (excluding Commissioned Officers in the uniformed services, and such special personnel systems as Foreign Service Officers, V.A. medical ranks, etc.) are blanketed into the SES. There are 10,777 SES slots for the Government as a whole; HEW has 780 of them. In theory the total number can be increased by Act of Congress, on request of the OPM made not oftener than every two years, but this is obviously a long and slow process.

4. Intramural scientists at NIH/ADAMHA who are qualified for supergrade appointments ordinarily exercise enough supervision over a supporting staff to qualify as "managers" for purposes of the SES. Thus when a scientist like Dr. Mortimer Mishkin, Chief of the Section on Cerebral Mechanisms of the Laboratory of Neuropsychology, is judged qualified for promotion above his present grade of GS-15, the natural course of action would be to promote him into the SES. If he is denied entrance into the SES he is in effect denied promotion.

5. I am informed by members of ASPER staff that OPM does not recognize "gradual assumption of duties" as justification for entry into the SES. OPM is reported to insist that before any new appointment can be made to the SES there has to be a full national recruitment effort. It is not clear to me whether OPM is saying that such a recruitment effort should be made for a non-existing vacancy, with the hope that the deserving incumbent can then justifiably be admitted into the SES, or that (a) only candidates who survive a national search can qualify for SES, and (b) since in the instant case there is no vacancy to recruit for and since a deceptive recruitment effort is unthinkable, therefore the scientist in question cannot get into the SES without successfully besting the competition for a real vacancy. In other words, he cannot continue in his present position and get into SES.

III. Implications

1. I would mention first the simple and I think noncontroversial fact that in programs like the NIH/ADAMHA intramural research programs the availability of career patterns for scientists is essential. Though not all scientists spend full careers here, nor should they, it is important to most of them to see that our structure and policies be arranged to reward high scientific productivity without artificial barrier. Indeed, a prime feature of the CSRA is its emphasis on reward for merit or performance. And yet the OPM policy now appears to be saying that regardless of the scientist's merit, he cannot continue in his present position and enter the SES. His present position often will be the only position which will enable him to carry out his research.

2. If the attributed OPM policy holds, our options would be the following: (a) play a reorganization game and try to develop a new position (officially a "vacancy") such that the scientist in question could handle it, and do the required search. It would still be deceptive, since no outsider could gain a position through it; (b) counsel patience for the scientist until an existing SES position became vacant, and for which he could then compete. For many there may well be no such positions, and in cases where there are, the wait may be insufferably long; (c) suggest that the scientist accept one of the outside offers of the many he normally will have received. In this case, as in the previous one, we lose a scientist.

3. Working scientists and scientist managers occupy a significant portion of SES slots in HEW. The appropriateness of including such scientists in the SES is recognized by approval of a "Scientific Service" as a subset of the SES. To recognize that scientists play an important role in the SES, and at the same time to deny the outstanding among them ready access to the SES is a contradiction hard to understand.

4. I am not aware that the CSRA forbids promotion into the SES by "gradual assumption of duties," nor do others who know the Act better than I believe it to be prohibited. The philosophy behind the SES does emphasize, quite rightly, the importance of merit in qualifying for entry into the SES. One approach to the assessment of merit is a competitive recruitment procedure. There are other methods, such as peer judgements of a scientist's achievements, which are also competitive, and which are as tight, fair, informed, objective, reliable and just, and which should be equally relied on for SES qualifications.

November 5, 1979

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Annual Report of the
Director, Division of Clinical and Behavioral Research
National Institute of Mental Health

October 1, 1978 - September 30, 1979

Robert A. Cohen, M.D., Ph.D.

In the annual reports of recent years I have commented on the general conditions under which we work, and have left to the laboratory and branch chiefs responsibility for describing their individual programs and achievements. This year their summaries again give eloquent testimony to the high quality and quantity of their investigations--at least they do to me and would, I believe, to a majority of scientists working in our field. This appraisal is based only in part upon my own judgment. It is corroborated by the regularly recurring evaluations of our Board of Scientific Counselors. It is further supported by the many invitations members of our staff receive to report on their latest work at meetings and symposia in this country and abroad; by the influence their methods and findings exert on the patterns of research in their respective fields; by the number of visitors who come to learn the techniques developed in our laboratories; by the requests for consultation and advice from investigators elsewhere; by the repeated efforts to recruit staff members for leadership posts at distinguished centers; and by the assortment of professional honors, awards and offices which come to those whom their colleagues regard as leaders in science.

By the foregoing comments I do not imply that for the past 26 years we have been moving ever upward from one peak of glorious achievement to another and yet another even higher. There were and continue to be management problems. The excitement of being in a young, vigorous and growing institution gives way to the burden of confronting the knotty issues faced by a mature organization. There comes a time when growth in size is no longer desirable or practicable. New directions require change, painful because it must be accomplished by rechanneling existing resources. Although everyone recognizes that research efforts worthy of the name must dare occasional failure, unanimous agreement on the time for termination is rare. A professional life devoted solely to full-time research was never possible even for the most gifted scientists of earlier generations. Now, such a career is open to many, and we are learning at first hand some of its vicissitudes as investigators endeavor to maintain creativity at an optimal level throughout the life course.

In the early years of the program these and other issues were considered and reconsidered as we shaped our management practices. The discussions were the concern of all, but they took place within the confines of the institution. The Congress and the Administration raised questions about the conduct of the work, but these were answered by the Directors most often without involvement of the researchers at the bench. In my view, it was the fact that so many vital decisions were reached in immediate direct interactions with the Director, NIMH and the Director, NIH that made this environment uniquely supportive of creative research. What was so refreshing in those early days was our freedom to institute whatever procedures we believed would foster the work and discard any which proved not useful. We were not confined by the usual constraints of institutional custom and convention.

Speaking parochially, I believe that there has been a gradual erosion of this desirable freedom to set our own goals. In my annual report last year, I stated "As program director, a significant amount of time once devoted to scientific issues is now directed to concern with the forms, regulations and administrative details which increasingly govern our investigative work." I pointed to the formal five year plan which is now carefully reviewed by higher authority beyond the Institute level, and to the newly instituted procedures for announcing and filling vacancies. Some proposals once acted on immediately must now be approved by authorities we never see, who never see us and who, in some instances, know nothing at first hand about the strengths and weaknesses of the setting in which we work. This year we have entered the newly-established Senior Executive Service which will govern our evaluations of our leading investigators, and set up a system of monetary rewards which, it is believed, will serve as reinforcement to improve creativity and productivity. The point I wish to make is that there are two ways for higher authority to change a system. One is to say, in effect, "We are not satisfied with this aspect of your current procedures. Develop others which take better account of these problems." The other is to say, "We are not satisfied with this aspect of your current procedures. Do it this way." We are moving toward the latter mode of operation.

I sometimes wonder how I would act if I were a Congressman charged with oversight of these research programs. The responsibility would not be easily discharged. The nation is approaching a financial crisis, but, like every other special interest group, each year the researchers ask for more. They declare that their work is flourishing, that they are on the verge of a break-through, of achieving a fundamental understanding which will ameliorate or possibly even relieve the suffering of millions of unfortunate individuals the world over. They speak in the same superlatives heard when one tunes in the television news, only to have the reports interrupted every few minutes by a persuasive individual extolling a breakfast food, a personal deodorant, a pain-killer or a detergent. The daily paper makes it painfully clear that we have moved from an Age of Heroes to an Age of Scoundrels. One learns of this irregularity or impropriety in meeting travel, in the awards of contracts, or of that seeming superficiality in the selection of areas of research. And such progress as has been made in the understanding and treatment of disease has driven medical costs to astronomical levels. In these circumstances, scientists can hardly expect to be freed from the system of checks and balances to which others are subjected. It certainly seems most appropriate to request that they employ the management techniques on which large private enterprises base their success. They must learn to be cost-effective. And since we cannot afford to cure disease, they should embark on a major campaign of disease prevention. So the researchers are advised to "do more with less," to develop a "can do" attitude, to "work smarter"; "when the going gets tough," they are reminded, "the tough get going." There is no doubt that such qualities in its citizens have served th nation well in times of adversity.

But there is another side to the issue. Almost 100 years ago, G. A. Tucker conducted a survey of the world's leading mental hospitals. A large majority of the superintendents declared that no institution should have more than 200 patients. Kirkbride, a leading figure in American psychiatry, declared that the hospital should be just so large that the superintendent could see every patient every day. Tucker remarked rather wryly that those few superintendents who advocated institutions large enough to serve as many as 400 to 500 patients seemed to find their greatest personal satisfaction in commanding a group of subordinates. In the face of this widespread conviction, one can only assume that pressures to increase the size of mental hospitals until eventually they became the shame of the states and of our society must have been responded to not with resistance based upon knowledge of need but with a "can do" attitude, a determination to "work smarter" and to "do more with less."

It is a truism that the promise of rewards can induce people to perform acts they would otherwise avoid. But there has been some research which indicates that monetary rewards may lead to poorer performance when the task is intrinsically interesting and requires inventive mental processing. In my opinion, it is just these conditions which obtain in our intramural research programs. The Government's own comparability pay figures are ample proof that our leading scientists are paid considerably less than their entitlement. They stay and continue to work in this setting because their value systems attribute greater importance to the positive advantages which this remarkable research institute still possesses. Whenever and for whatever reasons they come to believe that greater financial rewards are necessary, they move easily and quickly to the desirable academic posts which are repeatedly offered them. I am not saying our salaries should not be increased; over the years, successive Congresses and Administrations have presided over a modest but steady decrease in the buying power of our salaries. Perhaps the new system is intended to redress this situation but only for those selected as most deserving. It seems to me, however, that the good intentions which led to the institution of the Senior Executive Service inadvertently constitute an assault on the value system upon which our past success has been based and under which we continue our efforts. In a recent article, Freeman Dyson described that value system very well:

> "Much of the joy of science is the joy of solid work done by skilled workmen...There is a great satisfaction in building good tools for other people to use...The essential factor that keeps the scientific enterprise healthy is a shared respect for quality. Everybody can take pride in the quality of his own work and we expect rough treatment from our colleagues whenever we produce something shoddy. The knowledge that quality counts makes even routine tasks rewarding."

I cannot even conceive of a situation in which the new reward system will signigicantly improve our performance as scientists, and I fear that it may ultimately lead to disharmony and to a result opposite to that intended.

With respect to the importance of carefully articulated, long-term plans, one might call attention to the recent remarks of Sir Peter Medawar:

> "Following the modern fashion in funding research on a customer-contractor basis, if somebody asked: What, pray tell, is the genetic basis of the manifest differences in susceptibilities to multiple sclerosis and juvenile diabetes? How would you answer them?...By the ordinary processes of scientific research...faulty, expensive, long-winded and indirect...the differences came to be recognized and defined...This makes nonsense of the idea that discovery can be premeditated."

Medawar is a Nobel Laureate, but this lends only limited authority to his views since Nobel laureates can be as prejudiced and insensitive to truths as others less distinguished. But he is also Head of the Division of Surgical Sciences of the Medical Research Council. Since we can assume that he puts his convictions into practice in carrying out the responsibilities of that office, it will be interesting to compare his achievements with ours, as we adopt the practices and attitudes he decries.

If I were to close my remarks at this point, they would convey too negative an impression. Scientists are not lordly creatures who aspire to dispose of whatever may be proposed by less knowledgeable masses over whom they hold sway. Many issues which possibly could only have been raised by those outside the body of science have lead to useful and important changes in the ways in which our work is conducted. And while I believe that some of the changes in NIH over the years have not been for the better, nevertheless there are few settings anywhere in the world that equal it as a place in which to spend a significant portion, or even all, of one's life as an investigator in the biomedical sciences. It is a place where one still can search for truth--not the theologian's Eternal Truth--but for a scholar's intermediate goals, a place where one can work securely and in accordance with Jacob Henle's eloquent expression that,

> "The day of the last hypothesis would also be the day of the last observation. An hypothesis which becomes dispossessed by new facts dies an honorable death; and if it has called up for examination those truths by which it was annihilated, it deserves a monument of gratitude."

Annual Report of the
Director, Division of Special Mental Health Research
National Institute of Mental Health
October 1, 1978 - September 30, 1979

Richard Jed Wyatt, M.D.

New Biology and New Psychiatry

During the last 25 years, research in biological psychiatry has focussed on the synapse, the location thought to be the Achilles' heel of the brain. The opportunity for error in communication at this site presented imaginative scientists with the substrate for many hypotheses of disease states. Yet, it was the introduction of neuroleptic drugs which further riveted that attention. The drugs dramatically improved the lives of psychiatric patients. Understanding their mechanism of action could perhaps lead to understanding the enigmatic biochemical make-up of mental disorders. How did these drugs produce changes? How did they alter synaptic function?

Understanding the synapse began with reserpine, psychiatry's first modern drug. Reserpine was shown to deplete the pre-synaptic neurons of catecholamines and serotonin by acting on the storage granule. It prevents the granule from vaccuuming released neurotransmitters, allowing them to be destroyed by monoamine oxidase within the neuron. Since reserpine was useful in treating schizophrenia, the hypothesis developed that schizophrenia might be related to too much of one of the neurotransmitters. The ability of minute amounts of the hallucinogen, LSD, to alter serotonergic function led briefly to the hypothesis that schizophrenia might be due to either too much or too little serotonin.

Meanwhile, the neuroleptics were being explored. Over the last 15 years, evidence was accumulating that these drugs act by toning down dopaminergic function. The dopamine hypothesis (really two hypotheses--(1) that the neuroleptics work by blocking dopamine function; and (2) that there is a functional excess of dopamine)--was bolstered by understanding drugs such as amphetamine and apomorphine. Amphetamine, which releases dopamine, can produce a paranoid psychosis virtually indistinguishable from paranoid schizophrenia. Apomorphine, which was used to treat schizophrenic patients at the turn of the century, seems in low doses to decrease neuronal firing, lending support to the notion of an excess of dopamine in schizophrenia.

Since depression was thought to be caused by too little norepinephrine or serotonin, the monoamine oxidase inhibitors were designed to inhibit MAO and make more neurotransmitter available at the synapse. The biogenic amine hypothesis of depression became much more secure when the tricyclic antidepressants were found to block the reuptake of norepinephrine and serotonin into the pre-synaptic neuron, making more neurotransmitter available to the post-synaptic receptor.

Lithium, a relative newcomer in the exploration of the synapse, seems to act by modulating serotonergic function, perhaps interacting with calcium. It may well be the most powerful of the neuropsychiatric drugs because when it works, it seems to leave almost deficit. Learning how lithium works will provide a great deal of information both about affective disorders and the normal workings of the brain.

During the last year, knowledge of how the benzodiazepines function has become available, primarily from work done in the Laboratory of Preclinical Pharmacology. These drugs appear to promote GABA activity at the synapse by displacing an inhibitor. Further understanding of this process will provide information on sedation, sleep, and anxiety.

While psychiatry will continue to advance through understanding and attempting to manipulate the synapse, some psychiatric investigators have turned recently to examining CNS morphology of schizophrenic patients, an important pre-World War II pastime, which dwindled with the 1950-1970 "New Synaptic Pharmacology." The current findings reveal that some chronic schizophrenics have dilated ventricles, enlarged cerebral sulci, and cerebellar vermis atrophy. We do not know whether these findings are causative or merely associated with schizophrenia. We do not know if the abnormalities are reversible. We do not know whether the abnormalities are atrophic or developmental in origin. We do know that these structural abnormallties are not caused by either drug treatment or ECT, rather than they are associated simply with a diagnosis of schizophrenia. Further investigation is essential to understanding these abnormalities.

If some forms of schizophrenia are produced by cerebral or cerebellar atrophy, then in all likelihood the drugs which alter synaptic function will not completely restore function. Investigators must learn more about structural deficit of the brain and explore the myriad possible causes of atrophy. In order to adequately pursue these matters, researchers in psychiatry must cultivate new skills. For example, very little neuroanatomical work is currently taking place in psychiatry. Most of what is now done is a vestige of work done three of four decades ago, or is more related to disciplines other than psychiatry. Since the actual parts of the brain or brain pathways that are important to psychiatry are still a matter of speculation, an exploration of the enigma seems awesome. Nevertheless, a beginning is necessary and a precedent has been set.

We have learned that in psychiatry it is essential not only to examine the static autopsied human, but also to study the active system. There are a number of ways to study an active system. Basic scientists have been able to physically isolate a problem, enabling them to control a great many variables. Neurogeneticists have found ways of doing this in tissue from humans. For example, fibroblasts and lymphocytes can be cultured outside the influence of the body and its environment using tissue culture techniques. Chemical processes can be studied without regard to the presence of drugs. An abnormal gene can be localized and studied in detail. The gene product can be produced in as great a quantity as needed and it can be cooked, twisted, and broken until it reveals its secrets.

To understand the major scientific problems facing psychiatry, a program must develop new talents and abilities. I believe the way to proceed is to continue to find people who can cut across disciplines using the techniques of the geneticist, biochemist, immunologist, pharmacologist, and anatomist. I would like to see two sections (one on cytochemistry, one on neurogeneticists) set up within the Division for such a purpose. This multi-disciplinary approach seems essential to gaining a full grasp of the complex nature of mental disorders.

ANNUAL REPORT OF THE BIOLOGICAL PSYCHIATRY BRANCH
National Institute of Mental Health
October 1, 1978 - September 30, 1979

William E. Bunney, Jr., M.D.

During the past year the Branch has continued its productivity. One manifestation of this is the publication again of over 100 papers. Furthermore, five members of the staff received national or international awards for their research efforts: Dr. Candace B. Pert, in the Section on Biochemistry and Pharmacology, was awarded the Arthur S. Flemming Award for her "outstanding achievement as a research scientist in the identification of receptors on nerve endings which are specific for morphine-like substances"; Dr. Nartraj Sitaram received the A.E. Bennett Award from the Biological Psychiatry Society for his paper entitled "Development and Use of Pharmacological Probes of the CNS in Man: Evidence of Cholinergic Abnormalities in Primary Affective Illness"; Dr. Elliot S. Gershon was awarded the International Anna-Monika Award for Psychiatric Research (Third Place) for his work on genetic factors in manic-depressive illness; Dr. J. Christian Gillin received the Commendation Award of the U.S. Public Health Service for "leadership and imagination in conduct of studies on sleep, memory and schizophrenia" and Dr. Robert M. Post was awarded the Administrator's Award for Meritorious Achievement from ADAMHA for his "notable scientific contribution toward understanding of the psychobiology of affective illness". Each of these investigators is to be congratulated for exceptional research.

In 1975 the Biological Psychiatry Branch was selected to participate in a World Health Organization effort in biological psychiatry. Ten nations are participating. This year the Branch successfully hosted the annual meeting of this group and completed preparations of the first project for which the Bethesda group was the lead center. This was a project on red-green color blindness coordinated by Dr. Elliot Gershon.

The research activities of the eight sections or units in the BPB are reviewed below.

Section on Psychogenetics

Dr. Gershon is Chief of the Section on Psychogenetics. The general investigative strategy in this section is to study, in relatives of patients, the biological and clinical characteristics found in major psychiatric disorders. To this end, the study of well state in these disorders is required since state-dependent variables are virtually useless in studying the transmission of specific biologic vulnerabilities. The studies attempt to reveal the independent characteristics of patients with affective disorders, whether these characteristics are inherited, and whether the inheritance of the characteristics is related to the inheritance of the psychiatric illness. To date 2,535 relatives of 162 patients with primary affective disorders have been diagnosed. Based on preliminary diagnosis, the distribution of illness in first-degree relatives of the first 86 bipolar patients is bipolar (I and II) - 5.9%; unipolar - 10.4%; schizophrenic and other psychotic disorders -

2.3%; sociopathy, drug abuse, alcoholism and sexual deviation - 5.2%; cyc thymic personality - 2.1%. These figures are suggestive of familial diso with multiple manifestations, since they are clearly higher than populatio prevalences.

As noted above, this year we have combined our data on color blindnes linkage with that of other collaborative centers in the Biological Psychia Collaborative Program of the WHO. One pedigree suggested linkage, seven pedigrees suggested non-linkage, and eight were indeterminate. It is concl that at the most only a small proportion of bipolar illness may be linked color blindness. In the well state studies, one strategy has been to look for genetic variation in drug response, which could be directly related to adrenergic neurotransmission. Previous pharmacogenetic studies in psychopharmacology have largely been devoted to inheritance differences in drug metabolism as opposed to genetics of physiologic or psychological response drugs. Results with 13 monozygotic twin pairs and 3 dizygotic twin pairs using the pharmacological agent d-amphetamine shows that behavioral respon to this drug are likely to be heritable. These behavioral responses were correlated with amphetamine levels. This is the first demonstration of su a finding.

Section on Clinical Psychophysiology

This section is headed by Dr. Monte S. Buchsbaum. The section has focused its efforts on two major areas: opiate/endorphin modulation of pa and pharmacological alterations of attention. Using somatosensory evoked potentials and signal detection psychophysics, this unit, in collaboration with the Unit on Drug Abuse, was the first to report a hyperalgesic effect the opiate blocker, naloxone, in man. In a recent controlled and double-blind study it was shown that morphine produced the opposite of the specif evoked potential augmenting effect caused by naloxone.

In clinical studies, 17 patients with schizophrenia showed relative analgesia in comparison with age and sex matched normal controls as inferr from both psychophysical and evoked potential measures. Chronic naltrexon administration eliminated these differences in a subgroup.

By screening 400 male volunteers for attention, individuals at each extreme of a continuum have been identified. A very interesting subgroup individuals has emerged with an adult condition similar to childhood hyper activity and is being investigated further.

Unit on Drug Abuse

Dr. Glenn Davis, who has headed our Unit on Studies of Drug Abuse for the past four years, has accepted a position as Associate Professor of Psychiatry at the University of Tennessee. Dr. David Pickar, who has trained with Dr. Dennis Murphy, will assume administrative responsibility for this unit for at least a two-year period of time. Dr. Davis functione extremely well as head of the unit and has been extremely productive scient fically. The Unit has completed a number of studies utilizing narcotic antagonists such as naloxone and naltrexone in schizophrenia and during th manic and depressive phases of affective illness. Our original report in Science that there was a slight but statistically significant effect of the narcotic antagonist naloxone on psychotic symptomatology in schizophrenia was recently replicated. We have not found an effect of naloxone in depression and only one of 12 manic patients has responded to naloxone. We are currently conducting a study utilizing beta-endorphins in affective illness and schizophrenia in an attempt to test its possible effects on psychopathology in these two illnesses.

Section on Psychobiology

The Section on Psychobiology is headed by Dr. Robert M. Post. A number of areas are under investigation in this Section. A new procedure for life charting of manic and depressive episodes in patients with affective illness has been developed. A review of charts of six patients hospitalized for more than 25 years suggests that there is increased severity of illness over time, as manifested by increased frequency of manic-depressive episodes.

A major theme of the Branch which finds expression in most of the Units and Sections is the attempt to study neuronal receptor function in animals and in man. Apomorphine is a direct dopamine agonist which stimulates the dopamine receptors. The hypothermic effect of apomorphine was studied and has been reported for the first time in man. This approach may offer a direct measure of dopamine receptor responsivity in normals and in patients and when utilized with other indices, such as neuroendocrine responsivity, may give an index of dopamine receptor function in different areas of brain.

Work is continuing with carbemazepine. It has been shown that carbamazepine has unequivocal antimanic effects in some patients, including previous lithium nonresponders, as evidenced by improvement on drug and relapse on placebo substitution. We have also accumulated evidence that this compound may have an effect on dopaminergic mechanisms and this may, in part, relate to its mode of action. A pioneering study utilizing low dose piribedil and clonidine in manic patients is continuing, with positive results in terms of the antimanic effect of low-dose piribedil (ET495). After a long delay due to technical reasons, measurements of amine metabolites, enzymes and electrolytes in the cerebrospinal fluid of 44 normal volunteers is being undertaken, so that these normal volunteers can be utilized as controls for a large group of patients who have been studied. The world literature in this area is striking for its lack of normative data. Confirming previous work, we have demonstrated that urinary free cortisol is higher in the depressive phase of the manic-depressive illness than during the manic phase and that the changes in urinary free cortisol appear to be closely linked to the switch process into and out of mania.

Collaborative studies continue in the area of kindling in animals. Recent studies in collaboration with Drs. Charles Kennedy and Dr. Louis Sokoloff demonstrate that lidocaine-kindled seizures produce marked increases in glucose consumption in limbic areas including amygdala, hippocampus septum, and entorhinal cortex.

Unit on Sleep Studies

The Unit on Sleep Studies is headed by Dr. J. Christian Gillin. In collaboration with Dr. Gershon's group, a very important cholinergic REM induction test has been developed. Studies with this test suggest that euthymic patients with primary affective illness may have muscarinic supe sensitivity. If confirmed in future studies, this would be, to my knowle the first demonstration of a well state biological defect in affective ill

Studies in collaboration with Dr. Weingartner suggest that cholinerg mechanisms may play an important role in memory and that cholinergic drugs may be developed to aid patients with memory disorders. Studies, in colla tion with Dr. Wyatt, continued to investigate the role of neuropeptides in sleep and suggest that arginine vasotocin (AVT) may play an important role

Section on Neuropsychopharmacology

This section is administered by Dr. Daniel P. van Kammen and focuses primarily on psychobiological studies of schizophrenia. In contradiction the dopamine hypothesis, amphetamine induced brief improvement in 14 of 46 schizophrenic patients and produced no change in another 14 of the 46 pati Only 18 had an exacerbation of symptoms, as predicted by the dopamine hypo sis of schizophrenia. Preinfusion CSF MHPG levels correlated moderately w change in psychosis, but dopamine metabolites did not. Chronic administrat of the dopamine receptor blocker pimozide did not block the d-amphetamine effects. Response to d-amphetamine infusions seven days after pimozide wi drawal did not induce more intense behavioral effects. Both results were also contrary to predicted changes. As a group schizophrenic patients were more pain tolerant than normals. This pain tolerance was reversed by nalt one. Lithium was shown to have some minimal antipsychotic effects in some schizophrenic patients and some antidepressant effects in post-psychotic depression.

The hemodialysis project in schizophrenia continues. The amount of effort to conduct this study has been tremendous and so far only five pati have completed the 10 weeks on active dialysis and 10 weeks on sham dialys It is most important that this section complete a double-blind, crossover, sham-controlled trial of hemodialysis, because dialysis is currently being used in many hospitals throughout the world to treat schizophrenia althoug no scientific evidence to date documents its efficacy.

Unit on Childhood Mental Illness

During the past year, Dr. Judith Rapoport has been appointed Chief of the Unit on Childhood Mental Illness. This unit focuses on investigations biological factors in behaviorally disturbed pediatric populations and thos at risk for later psychiatric disorder, primarily hyperactive children and offspring of patients with affective disorders The unit has demonstrated that the response of hyperactive children to stimulant drugs is not parado either with respect to normal children or to college age adults. One of th most effective drugs in the treatment of hyperactive or minimal brain dysfunction children is amphetamine. A series of pharmacological studies has demonstrated for the first time that the clinical response to amphetamine occurs primarily during the absorption phase rather than the elimination phase of drug metabolism. This implies that it is saturation of the receptors rather than plasma concentration per se which may be important for clinical response.

It has been hypothesized that the neurotransmitter dopamine may be specifically involved in hyperactive behavior in children. However, the use of two drugs which specifically affect dopamine metabolism, piribedil and Sinemet, gave negative results, thus arguing against selective dopaminergic activity as the basis for the primary efficacy of stimulant drugs in hyperactivity.

A pilot study of lithium treatment of behaviorally disturbed offspring of lithium-responding parents was undertaken to test the hypothesis that these individuals might specifically respond to lithium. Preliminary data suggests that a variety of behavioral disturbances, in addition to affective illness, may be responsive to lithium therapy in this particular population. This finding may eventually suggest a new means of categorization of childhood disorders on the basis of pharmacological response and may provide a supplement to clinical descriptive classification.

Section on Biochemistry and Pharmacology

The Section on Biochemistry and Pharmacology is involved in a number of projects directed toward understanding how the brain modulates the sensitivity of its response to neurotransmitters. Studies of receptors in culture using HeLa cells have demonstrated that the "coupling" is a separate event from increase in beta-adrenergic receptor number. Phospholipid synthesis is under beta-adrenergic control and an increase in phosphatidylcholine synthesis occurs in the presence of beta-agonists.

The effect of lithium on the development of supersensitivity and neuronal receptor systems has clearly been more extensively studied in this laboratory than in any other laboratory. A number of studies of lithium's effects on the development of super-and subsensitive neuronal receptors are underway. It has been shown that lithium can block the development of supersensitivity in beta-adrenergic receptors following 6-hydroxydopamine. Altered response to cholinergic agonists has been demonstrated after lithium treatment. However, lithium does not seem to block either the beta-adrenergic receptor subsensitivity or the electrophysiological supersensitivity response to serotonin after tricyclic antidepressants.

It has been postulated for some time that there is an interaction between the GABAergic system and the mechanism of action of the minor tranquilizers. The direct evidence for this extremely important observation was first reported by investigators in this section. It was clearly demonstrated that altering GABA affected the binding of the benzodiazepines. Activation of the GABAergic system renders the brain more sensitive to the benzodiazepines. It is possible that benzodiazepines alter neuronal inhibition in the CNS.

Work on new peptide hormone receptors for vasoactive intestinal polypeptide (VIP) and bombesin was first reported by investigators in this group. The distribution and immunoreactivity of bombesin-like material suggest the possibility that bombesin may be a transmitter. Utilizing a radioreceptor assay and radioimmunoassay, beta-endorphin is being studied in CSF and plasma. An increase in beta-endorphin has been observed in female rats in labor. Behavioral studies show that descending noradrenergic and serotonergic pathways appear to mediate beta-endorphin analgesia.

Annual Report of the Clinical Neuropharmacology Branch
National Institute of Mental Health
October 1, 1978 to September 30, 1979

Dennis L. Murphy, M.D., Chief

During its third year of existence, the Clinical Neuropharmacology Branch has continued to direct the majority of its research effort towards (1) clinical studies of the biological and behavioral effects of antidepressant drugs and (2) studies of some neurobiologic systems which seem likely to represent junction points between affective disorder symptomatology and sites of antidepressant drug actions.

The clinical research unit on 3-East remains the locus of the Branch's greatest efforts. A great deal of credit for the success of our studies in depressed patients and volunteers on the unit is due to major collaborative contributions from other NIMH and NIH groups. More directly, it is due to the highly able and enthusiastic support from the nursing and other clinical staff on the unit, guided in the last year by our ward administrators, Robert M. Cohen, David Pickar and Larry Siever, who served in rotation. Our off-ward clinical studies include examination of longer-term drug effects in a small outpatient group, and a larger scale study of the psychological and biological parameters which may predispose apparently normal individuals to a higher-than-normal risk of mental disorder.

Most aspects of our comparative study of two monoamine oxidase (MAO)-inhibiting antidepressants, clorgyline and pargyline, were completed during the past year. This study was a major attempt to evaluate the therapeutic potential of selective inhibition of either MAO type A alone (by clorgyline) or of MAO type B alone (by pargyline), and to characterize the biological and psychological consequences of selective inhibition of each of the two forms of the enzyme. Currently-available MAO inhibitors are used infrequently in the treatment of depression, partly because they are thought not to be as broadly effective as tricyclic antidepressants across all groups of depressed patients, and partly because they induce a number of undesirable side effects.

In our study, it was possible to verify that clorgyline produced marked MAO-A inhibition in patients, and that its selectivity for inhibiting the MAO-A enzyme without inhibiting MAO-B was maintained over the four week clinical trial period. Indices of MAO-A inhibition included measurement of the deaminated metabolites of two MAO-A substrates (norepinephrine and serotonin), whose concentrations were substantially reduced in cerebrospinal fluid during clorgyline treatment. Direct measurement of platelet MAO-B activity indicated that this enzyme remained uninhibited. Although pargyline almost completely inhibited the MAO-B activity in platelets, it also partially inhibited MAO-A activity. Pargyline, however, produced greater effects on the metabolic products of dopamine and phenylethylamine, two substrates which are primarily deaminated by MAO-B in man.

The results from the behavioral assessment measures during the four week, double-blind, random-assignment study of clorgyline compared to pargyline in sixteen depressed patients yielded clear evidence that the two drugs had different clinical effects. In the original study of comparative efficacy set up by Dr. Lipper, the patients receiving clorgyline all demonstrated reduced Hamilton depression scale ratings, with a mean difference that was statistically significant. Similarly, antidepressant and antianxiety effects, and improved functional capacity, were reported by the clorgyline patients, with al six subscales of the NIMH self-rating scale yielding statistically significant differences. In contrast, the patient group receiving pargyline remained essentially unchanged during the period of treatment. There was no indication of improvement on the Hamilton scale and little evidence for change on the NIMH self-rating subscales.

Side effect of all types in the crossover study were more frequent and more severe in the pargyline-treated patients. Hypomania and postural hypotension occurred with equal frequency with both drugs. No hypertensive episode occurred. However, clorgyline treatment was found to be associated with enhanced sensitivity to intravenous tyramine administration in the studies conducted this year by Dr. Pickar, indicating that susceptibility to a hypertensive response during clorgyline administration remains a liability of this drug, and a lesser problem with the MAO-B inhibitors pargyline and deprenyl.

Of several possible interpretations of these results, it seems most likely that relatively selective changes in norepinephrine and/or serotonin metabolism could be postulated as the basis for the antidepressant effects in the clorgyline-treated patients. Such an interpretation would agree with evidence demonstrating that selective MAO-A inhibitors are more effective in reversing the sedative and locomotor effects of reserpine or tetrabenazine in one of the most widely used psychopharmacological animal models of depression. Thus, it may be that inhibition of MAO type A is more closely related to the antidepressant effects of MAO inhibition than is MAO type B inhibition.

In other studies of patients with affective disorders, acute activation and euphoriant responses to replicated single day trials with d-amphetamine proved to have moderately high correlations with subsequent antidepressant responses to imipramine and lithium carbonate during 3-4 weeks of treatment. These studies suggest that common factors underly responsiveness to different drug groups in individual depressed patients. Preliminary data using Minnesota Multiphasic Personality Inventory sub-scales indicated that predictive estimates of antidepressant responses to lithium and imipramine may also be obtained from pretreatment psychological tests. An extensive review of the question of the prediction of drug responses and the relationship of drug responses to diagnosis was completed in conjunction with Dr. Robin Murray of the Maudsley Psychiatric Institute during his sabbatical with our group a year ago. In a detailed, longitudinal case study of a cycling bipolar patient conducted by Dr. Pickar, changes in tyramine pressor responsivity were observe to correspond to changes in depressed vs. hypomanic behavior, raising speculation that such clinical state changes may involve changes in receptor sensitivity--a possibility under continued study. Additional areas of investigation in patients with affective disorders include studies of personality features and of symptoms such as anxiety in subgroups of depressed patients, conducted in collaboration with Dr. Donnelly. Studies of memory and cognition in relation to mood state and drug effects are being conducted by Dr. Robert M. Cohen in collaboration with Dr. Weingartner. Dr. Hoover has continued to investigate marital conflict and dominance issues in patients with affective

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disorders compared to community controls. A series of projects to evaluate the assessment instruments and rating scales used in neuropharmacologic research in the affective disorders have been set up this year; a review of this area was recently completed.

In investigations of patients with alcoholism organized by Dr. Major, studies of clinical symptomatology in young healthy male alcoholics demonstrated a lack of the association between depression and alcoholism which has previously been reported. The alcoholic group was also found to have no more aggressive behavior than age-matched normal controls, although the alcoholic group had a more aggressive attitude. Platelet monoamine oxidase was lower in alcoholics. Disulfiram, which is used as a regular part of alcoholism treatment programs, caused an increase in both serum cholesterol and plasma norepinephrine, with an associated increase in blood pressure, raising the possibility that long-term disulfiram use might increase the risk of hypertension and/or cardiovascular disease. Disulfiram also precipitated psychosis in eight individuals who prior to taking disulfiram had lower platelet and plasma amine oxidase activities, higher erythrocyte catechol-O-methyl transferase activity, and lower cerebrospinal dopamine-β-hydroxylase activity. Autopsy brain samples from alcoholics contained a greater number of GABA receptors and a lower number of β-adrenergic receptors than did control brains.

The major focus of our investigations of the relationship between endogenous MAO activity differences and behavior has shifted from patient groups to normal populations. In continuing studies of individuals in the upper and lower 10% of the distribution of MAO activity from a sample of 375 normals, rapid eye movement sleep and cortical evoked response differences have been shown to be associated with platelet MAO differences. Studies of factors capable of affecting platelet MAO have shown that smaller, lighter platelets have less MAO activity than larger, heavier platelets; however, most patients and normals with low MAO activity were not found to have a larger proportion of small, light platelets than controls. Marijuana smokers have lower platelet MAO activity than controls, but short-term tetrahydrocannabinol administration or its addition to the enzyme in vitro did not reveal any significant inhibitory effect. These findings, in general, are consistent with our previous observations in patients and normals suggesting that reduced platelet MAO activity is associated with differences in personality features, psychobiological characteristics and a tendency towards increased vulnerability to psychopathology. Several new populations in normals in which a higher prevalence of these personality characteristics might be expected are now under study. Similarly, a new variable, smooth pursuit eye tracking impairment, has begun to be investigated in normal populations by Dr. Siever, using the same biological high risk strategy originally developed for the platelet MAO activity studies in normals.

In laboratory investigations, Drs. Campbell, Cohen and Major have been employing a variety of approaches in rodents, cats and monkeys to evaluate adaptive mechanisms during the acute, and more especially the longer-term administration of psychoactive drugs. MAO-inhibiting drugs, tricyclic antidepressants, alcohol, disulfiram, and several groups of receptor agonists and antagonists have been investigated most extensively, and these studies have provided information rendering our clinical studies more easily interpretable. In particular, the development of methods for the periodic or continuous sampling of cerebrospinal fluid in cats and monkeys, and the availability of autopsy specimens fr VA hospital patients, has helped bridge some of the gaps between our clinical neuropharmacology studies and studies of drug effects in rodents.

Dr. Costa has continued innovative work with the platelet as a model for the study of events related to amine metabolism at the cellular level. The processes of amine uptake and amine storage, and the number and composition of platelet storage vesicles have been examined utilizing radiolabelled tracers, transmission electron microscopy, and electron microprobe X-ray analysis. Synchrotron radiation was used to study the fine structure of the calcium in various model systems. The studies have three principal objectives: (1) quantitation of amine uptake, and of the number and composition of storage vesicles in platelets following treatment with various psychoactive drugs and releasing agents; (2) evaluation of the mechanisms responsible for amine movement across membranes; (3) examination of the chemical and physical environment of storage vesicles. Other studies employed electron energy-loss spectroscopy to document the ultrastructural location of fluorinated molecules inside platelets and synaptosomes. The data provided information about the intracellular sequestration of fluorinated analogs of biogenic amines. Developmental work on the role of radiation damage in limiting the information obtainable, quantitation and image processing of elemental maps, and the possibilities for chemical analysis is being carried out. Intact platelets and platelet extracts have also been studied by biochemical analysis and by the nuclear-magnetic-resonance examination of fluorinated psychoactive drugs and amines and of phosphorylated compounds of low molecular weight. Some chemical and physical concomitants of drug, amine, and nucleotide storage have been determined, and correlated with differences in the composition of storage vesicles. In addition, lithium contained in model systems, platelets, and erythrocytes has been studied by nuclear-magnetic-resonance. The lithium resonance in model systems and cells appears to reflect the water content and relative motional restriction of the immediate environment.

In these studies, important species variations in the platelet amine storage mechanism have been found. Although platelets from different species demonstrate a similar capacity to store serotonin, the mechanism by which storage is accomplished appears to differ widely. Explorations using the platelet have provided opportunities not found in more complex tissues to define some of the physical and chemical bases underlying amine movement across the plasma and vesicular membranes, and for the sequestration of calcium, nucleotides, and serotonin inside platelet vesicles.

Annual Report of the Clinical Psychobiology Branch
National Institute of Mental Health
Frederick K. Goodwin, M.D.

During the past year, the Clinical Psychobiology Branch has continued to develop in several new areas, while maintaining momentum in a core of ongoing long-term studies.

Dr. Steven Paul joined the Branch in 1978 as a Clinical Associate, after two years in Dr. Axelrod's laboratory. This year Dr. Paul has assumed a tenured position in the Branch as Chief of the Unit on Preclinical Pharmacology, which includes a Fogarty International Fellow, a NIMH Postdoctoral Fellow, and a technician. Dr. William Potter has accepted an appointment in the PRAT program of the NIGMS, but will continue to work in the Branch on a joint appointment. In the Branch he will serve as Chief of the Unit on Clinical Pharmacology, which includes a Fogarty International Fellow and two Research Associates (PRAT program). Dr. Rex Cowdry, who during the last year worked part time in the Branch on an I.P.A. agreement with Georgetown University, has rejoined us in a full time position as Chief of the Outpatient Unit. This unit has been strengthened by the recent addition of a full time secretary. Next year a full time Clinical Associate will be assigned to this unit. The Unit on Neuroendocrinology has been enlarged recently by hiring a full time Research Assistant.

(1) The Clinical Research Unit continues to serve as a major focal point for the research efforts of the Branch. Clinical studies of patients with affective and schizoaffective illness entail the integration of behavioral, biological, and pharmacological observations, often on a longitudinal basis. Work on methods for evaluating changes in mood states continues. The therapy program on the ward has been structured according to a group model with considerable emphasis given to the role of the primary nurse clinician. Behavioral data are collected at least twice daily on all patients. The core of the behavioral data collection system continues to be the global ratings arrived at by consensus of the nursing research team; individual item ratings such as the BPRS are done twice a week. Dr. Siwers from the Karolinska Institute was recruited on a temporary basis to conduct a cross ward reliability study of our rating systems. Motor activity (see Project No. Z01 MH 00450-05) and sleep (assessed at half hour intervals) are reported daily on all patients. All drug trials are conducted under double-blind conditions.

(2) The study of biological rhythms continues to be a major focus of the Clinical Research Unit. Our underlying hypothesis is that the inherent cyclicity of manic-depressive illness (i.e., an abnormal, long term rhythm) is in some way related to disturbances in the regulation of shorter term rhythms, particularly circadian (24-hour) rhythms. Activity, sampled continuously on all patients every fifteen minutes using the self contained ambulatory activity monitor. is the major behavioral data base for these studies. More than one week of continuous data can be stored in the new model monitor, completed this year, whose overall size is less than 1/2 a cigarette pack. Biological measures include temperature, sleep EEG, urinary MHPG, plasma melatonin, and a variety of hormones. Major findings this year include:

(a) Further evidence supports our previous hypothesis that diurnal activity rhythms in bipolar patients are not synchronized with the environmental light-dark cycle.

(b) Compared with controls, bipolar depressed patients have an earlier peak in MHPG excretion and in temperature (i.e., are phase advanced).

(c) Eight out of ten bipolar patients, monitored continuously, experienced 48-hour (bicircadian) sleep-wake cycles at the time of a switch into mania. A similar pattern was observed in normals after prolonged "free running" exposed to continuous light, isolated for time cues.

(d) Rhythm studies of patients with primary insomnia (difficulty falling asleep) indicate that they, in fact, have a phase delay in their sleep-wake cycle rather than a decreased ability to sleep. A treatment strategy involves progressive delay of sleep phase until a "normal" schedule is reached, that, remarkably, is then maintained.

(e) In free running hamsters studied in a new animal chronobiology facility (under the direction of Dr. Anna Wirz-Justice) chronic tricyclic treatment was shown to accelerate the intrinsic periodicity of the hypothalamic clock for the rest-activity cycle. This finding may be relevant to our clinical observation that this class of drugs can accelerate the cycle in manic-depressive patients.

(f) Following up on our observation that patients switching out of depression frequently evidenced a phase advance in the activity-rest cycle, we undertook experiments in which patients were artificially phase advanced following one night of sleep deprivation. The first patient placed on a six-hour phase advance sleep schedule (going to sleep at 5 p.m. and awakening at midnight) showed a dramatic remission of her depression within two to three days, a remission that lasted about two weeks. The remission was reproduced by a second phase advance of six hours (going to sleep at noon and waking up at 7 p.m.) Further advances, however, did not reproduce the earlier remission. Two of three additional patients showed a partial improvement with this phase advance technique. The relationship between the clinical effects of phase advancing and the clinical effects of sleep deprivation are under investigation.

(g) Examination of seasonal rhythms in CSF amine metabolite data indicate that the probenecid-induced accumulation of both HVA and 5HIAA evidence a significant seasonal rhythm, with a low point in spring and a peak in autumn; the norepinephrine metabolite, MHPG, shows the opposite seasonal rhythm.

(h) Our earlier observation of rapid cycling induced by maintainance tricyclic antidepressants in bipolar patients has been extended. Eighteen of 26 bipolar patients experienced manic or hypomanic episodes on tricyclics, and the latency from the beginning of drug administration to hypomania/mania showed a strong and significant positive correlation with pretreatment MHPG; this finding is supportive of a model based on differential noradrenergic receptor sensitivity.

(i) Diurnal rhythms in the activity of α- and β-adrenergic receptors (involving changes in receptor number) have been identified and chronic treatment with imipramine has been shown to alter the α-adrenergic rhythm, producing a marked 12-hour phase shift. This drug induced a more modest 4-hour phase shift in the β-adrenergic rhythm. A circadian rhythm in striatal dopamine receptor was also demonstrated, with peaks in the mid-light and in the mid-dark phases.

(3) Melatonin studies in humans: Our interest in the pineal gland continues, not only because of its role as an *in vivo* marker for the biological clock regulating the activity-rest cycle, but also because it provides an *in vivo* measure of the activity of a well-characterized β-adrenergic receptor. Human pineal studies have been limited by difficulties in the measurement of the small amounts of the pineal hormone, melatonin, that appear in plasma. Using negative ion gas chromatography-mass spectrometry, Dr. Lewy, in collaboration with Dr. Markey in the LCS, has perfected a new method with high specificity and sensitivity. This method permits the accurate and reproducible measurement of the small amounts of melatonin occurring in human plasma. Major melatonin findings this year are as follows:

(a) In normals, plasma melatonin increases substantially at night, and falls rapidly at dawn, staying at very low levels throughout the day. In manic patients, the 24-hour mean excretion of melatonin is higher with some suggestion of a phase advance, that is an earlier occurrance of the nighttime peak. Bipolar depressed patients have significantly lower melatonin secretion than do manics.

(b) Artificial light of normal intensity does not effect melatonin secretion in man, unlike any other animal species. Sunlight and artificial light of extremely high intensity do suppress melatonin in man.

(c) Plasma melatonin has been shown to correlate with sleep stages in normals, with bursts of melatonin occurring during various stages of sleep.

(d) In patients and in normal controls, propranolol (a β-receptor blocker) reduces melatonin secretion at night and the extent of reduction shows a precise dose response relationship.

(e) Chronic tricyclic antidepressant treatment in animals reduces the isoproterenol-induced increase in daytime melatonin by 20%, while nighttime melatonin secretion is reduced by more than 50%; these findings indicate that both pre- and postsynaptic noradrenergic function is reduced by chronic tricyclic administration.

(4) Cerebrospinal fluid amine metabolites in mental illness: These long-term studies in a variety of psychiatric illnesses continued this year. The Clinical Psychobiology Branch serves as the coordinating unit for CSF studies in the division, and works collaboratively with various laboratories, including the Laboratory of Clinical Science. This year has seen a major effort to obtain CSF from large numbers of hospitalized normal controls, a project conducted in collaboration with Drs. Post and Ballenger in the Biological Psychiatry Branch. Major findings this year include:

(a) Chronic DMI treatment of depressed patient reduced CSF MHPG but not 5HIAA while chronic treatment with zimelidine, a relatively specific serotonin reuptake inhibitor, reduced CSF 5HIAA but not MHPG.

(b) CSF MHPG does not correlate with urinary MHPG. On the other hand, CSF norepinephrine does correlate with urinary MHPG.

(c) Within a group of alcoholic patients, low levels of dopamine beta-hydroxylase in the CSF were associated with a markedly elevated MMPI profile.

(d) Subjects with personality disorders, but without major psychiatric illness, were studied in collaboration with the National Naval Medical Center. Levels of the serotonin metabolite (5HIAA) in the CSF were found to correlate negatively with life history of aggression, while CSF MHPG showed a positive correlation with this behavioral measure.

(e) ECT was found to decrease levels of 5HIAA in the CSF. However, the most pronounced effect of ECT was a "normalization" of pre-existing levels of both 5HIAA and HVA; that is, low initial levels increased while high initial levels decreased. Similar "normalizing" effects were observed in patients receiving chronic lithium therapy. The relationship between this stabilization effect and the clinical effects of these treatments (i.e., effects in both mania and depression) is of interest.

(5) Urinary norepinephrine metabolites in affective illness and controls: This ongoing project evaluates a wide variety of factors that contribute to the urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG), the norepinephrine metabolite thought best to reflect brain turnover. Major findings this year are as follows:

(a) Within individual patients the circadian rhythm of urinary MHPG parallels the circadian rhythm of activity; however, between individual patients total 24-hour activity does not correlate with total 24-hour MHPG.

(b) In a replication of previous work, depressed patients with a prior history of mania (bipolar I) had significantly lower MHPG than normals, unipolar depressed patients, or manic patients, although the absolute differences are modest.

(c) The ability of low urinary MHPG to predict antidepressant response to imipramine has been replicated; the use of urinary MHPG to predict amitriptyline response is not as clear.

(d) Chronic treatment with DMI and zimelidine (drugs relatively specific for norepinephrine and serotonin, respectively) decrease urinary MHPG.

(e) Effects of monoamine oxidase inhibitors (type A and type B) on urinary MHPG have been studied in collaboration with the Clinical Neuropharmacology Branch; both types of inhibitors produce a substantial decrease in urinary MHPG, averaging 80%.

(6) Clinical and laboratory studies of morphine-like compounds: Interest in the role of endogenous opiate peptides, called endorphins, in central nervous system functioning has continued. Some aspects of our clinical work in this area have been conducted in collaboration with the Biological Psychiatry Branch. Much of our laboratory work has centered on the compound FK 33-824, a synthetic enkephalin analogue that is quite active when administered parenterally or orally and hence, may be of clinical use. Major findings this year in these studies are as follows:

(a) In rats, FK 33-824 produces inhibition of conditioned avoidance response, blockade of anxiety behavior, increases in serum prolactin, and apomorphine-like stereotyped behavior, all suggesting that the interactions of this enkephalin with brain dopamine and norepinephrine systems are complex.

(b) Preliminary clinical trials of the morphine-like compound, methadone, in affective illness indicate some anxiolytic and mood-elevating properties.

(c) The narcotic antagonist, naloxone, was found to have little or no effect in ten manic patients.

(7) Clinical pharmacology of antidepressants: These series of studies have represented a major new thrust stressing pharmacokinetics during the last year. They have been conducted in the new Unit on Clinical Pharmacology under the direction of Dr. William Potter. These clinical studies have depended on the prior development of a highly sensitive GC-MS assay for tricyclic antidepressants by Dr. Muscettola in our Branch, in collaboration with Dr. Markey in the LCS. This year our clinical pharmacology program has been enriched by the addition of a M.D.-Ph.D. Psychiatrist on a fellowship sponsored by the NIGMS Clinical Pharmacology Program (PRAT Program). A Ph.D. neuropharmacologist has also joined this program with a PRAT fellowship. Major findings this year are as follows:

(a) Our previously developed method for predicting ultimate steady-state tricyclic concentrations using single oral doses has now been applied in a routine manner to patients under treatment with desipramine. Accurate prediction of steady-state plasma levels can be made with this single dose model.

(b) Across patients, interindividual variation in plasma tricyclic concentration is lower than previously reported and shows considerable stability within an individual.

(c) Reported variations in red cell/plasma ratios of tricyclics are partially artifactual resulting from differential equilibration between these two tissues during dosing intervals.

(d) Imipramine in pre-pubescent boys is demethylated more actively than in adults.

(e) By measuring tricyclic concentration in the CSF (i.e., not protein bound), it has been demonstrated that total steady-state plasma concentrations provides a reasonable estimate of free drug; i.e., total plasma level correlates highly (r=0.94) with CSF concentration.

(f) Plasma drug levels in patients on the antidepressant zimelidine (a serotonin uptake inhibitor) indicate a very close relationship between the dose and the concentration of norzimelidine.

(g) Eight patients have completed a unique double-blind cross-over trial of an experimental antidepressant (zimelidine) and an established antidepressant (DMI). Zimelidine has failed to precipitate manic episodes or rapid cycles in bipolar patients, in contrast to DMI. There is no overall difference in the antidepressant efficacy of the two drugs, despite differential effects on norepinephrine and serotonin metabolites in the CSF.

(h) Steady-state concentrations of DMI correlate positively with decrease in MHPG in CSF, whereas such an association is not seen with urinary MHPG.

(i) The recent demonstration that hydroxylated metabolites of tricyclics are active in amine reuptake inhibition has been replicated. We have also shown that hydroxy metabolites can form a major proportion of the total tricyclic in the blood of some individuals, and are also present in the CSF in relatively high concentrations. Accounting for hydroxy-desipramine improves the correlation between drug concentration and clinical effect in children with enuresis.

(j) Accute and chronic administration of zimelidine and DMI to patients had no significant effect on plasma growth hormone, leutinizing hormone, prolactin, or urinary free cortisol.

(8) Neuroendocrine studies of major psychiatric disorders: The Unit on Neuroendocrinology under the direction of Dr. Philip Gold, serves as the major

neuroendocrine coordinating group for the Division. A major function of this Unit is the maintenance of collaborative arrangements with several leading university research facilities to provide neuroendocrine assays. Some of these assays are conducted here in our own laboratories, but resource limitations continue to make outside collaborations necessary. Affective illness has been the major focus of the neuroendocrine studies, principally because of the close association between its symptoms and hypothalamic function. The neuroendocrine strategies currently in use include the direct measurement in CSF and plasma of behaviorally active peptides, with or without stimulation; infusion of dopamine and administration of various hypothalamic releasing factors to test the responses of the hypothalamic pituitary axis; study of the effects of psychoactive drugs on hypothalamic function and on the levels of behaviorally active peptides. Major findings this year are as follows:

(a) The role of the central nervous system peptide, vasopressin, in affective illness has been hypothesized in a major review, and this hypothesis is now under experimental evaluation. Levels of vasopressin in the CSF are significantly higher in manic patients compared with depressed patients or controls; depressed patients show a trend towards lower values than controls.

(b) Plasma vasopressin is known to increase following the infusion of hypertonic saline in normals, a phenomenon that is highly stable and reproducible in the same individual and is heritable. Manic patients show greater sensitivity of the vasopressin response to hypertonic saline compared with bipolar depressed patients. This finding in mania is compatible with our initial hypothesis of relatively augmented vasopressin function in this state. Lithium, a vasopressin receptor blocker, significantly augments the vasopressin response to hypertonic saline, while carbomazepine, a vasopressin receptor agonist, significantly reduces vasopressin response to hypertonic saline.

(c) A double-blind placebo controlled trial of the vasopressin analogue DDAVP has been undertaken in patients with major affective illness. Preliminary findings show that this analogue induces highly significant improvement in measures of memory, and in some patients, this is accompanied by significant amelioration of depressive and psychotic symptoms.

(d) In normal subjects, a significant positive correlation was found between CSF vasopressin and test of memory function.

(e) In collaboration with the LCS, vasopressin function was shown to be diminished in patients with anorexia nervosa.

(f) Preliminary findings indicate that schizophrenic subjects have elevated levels of the peptide angiotensin I in their CSF compared with normals.

(g) CSF somatostatin levels are higher in manic patients than in bipolar depressed patients.

(h) Dopamine infusions in patients with affective illness, schizophrenia, and anorexia nervosa, have been studied in conjunction with similar studies in normal controls. In normals, dopamine infusion produces a drop in LH, followed by a rebound. Drug free schizophrenic patients show significantly greater LH suppression with no rebound, while drug-free manics show a paradoxical LH rise during the infusion. The findings in schizophrenics suggest hypersensitivity of some hypothalamic dopamine receptors. LH responses to dopamine infusion are increased following withdrawal of pimozide, a dopamine receptor blocker. This is in contrast to prolactin responses that are diminished during the pimozide withdrawal period. Patients with anorexia nervosa show a blunted LH response to dopamine infusion. Plasma dopamine levels decrease progressively during the course of a dopamine infusion, a finding that may be secondary to the increased plasma and platelet monoamine oxidase observed.

(i) Urinary free cortisol has been found to differ among subgroups of affective illness, being highest in unipolar patients with psychotic features, and lowest in patients in the manic phase. Among unipolar patients, the urinary free cortisol showed a significant positive correlation with CSF 5HIAA.

(j) Relationship between neuroendocrine function and sleep has been studied in collaboration with Dr. Gillin, and details can be found in the report of the Biological Psychiatry Branch.

(9) Outpatient follow-up studies of manic depressive patients and their families: Ongoing long-term follow-up studies of manic depressive illness continued this year, focusing principally on psychodynamic issues and the interactions between patients and their families. The primary source of information for these studies has been the group treatment modalities that serve the dual purpose of treatment and research. In addition, this year the outpatient research staff completed a major analysis of longitudinal data on several hundred manic-depressive patients studied in collaboration with Dr. Paul Grof.

Two couples groups comprising index patients and their spouses, meet weekly and provide a basis for studying the relationship between psychotherapeutic modalities and long-term outcome in bipolar patients maintained on lithium. In addition, several of our patients are members of families in which affective illness has been manifest for three or more generations, allowing for the exploration of familial transmission patterns, some of which may reflect non-genetic factors. Some major areas of investigation this last year are as follows:

(a) In collaboration with the Psychogenetics Unit of the BPB, attitudes and perceptions of spouses towards manic-depressive illness were examined. It was found that unfavorable attitudes towards the illness were significantly more likely among spouses than index patients, particularly with respect to the impact of the illness on the marriage.

(b) Six couples (one member with bipolar illness) were followed through the course of a pregnancy and birth. Two of the three female patients who were taken off lithium during pregnancy developed post-partum mania, and one bipolar husband developed post-partum mania. Special psychodynamic issues associated with impending parenthood among bipolar patients have been described and are being published.

(c) The six male infants born to these couples are participating with their parents in a study of the development of infants at risk for bipolar illness; all relevant variables are studied in matched controls as well.

(d) In collaboration with Dr. Paul Grof, we completed an analysis of the course of illness in 334 unipolar and bipolar patients followed an average of 13 years without maintenance treatment. Fifteen percent of the bipolar patients did not have their first mania until after the third episode of illness, and therefore would have been falsely classified as unipolar. In both the unipolar and bipolar groups, age of onset was positively correlated with the time between the first and second episode. Thus, age of onset may be helpful in determining which patients should be placed on prophylactic treatment following their first manic episode. In this large sample, cycle length was found to decrease with each succeeding episode until the fourth episode, following which the rate of acceleration in the illness declined; the rate of decrease in cycle length was not significantly different between the unipolar and the bipolar groups.

(10) Brief outpatient-inpatient studies of depressive syndromes: This is a ne program, currently in its pilot phases. The program explores feasibility of collaborative-consultative research and treatment involving outpatients referre by private practice psychiatrists in the area, and also involving continuity o care with the private physician. Preliminary experience with the first ten patients indicates that the program is workable, and has been of significant clinical benefit to all but one of the patients. There are major advantages t the collaborative model, particularly in that it does not require separating th patient from his referring physician.

The project has undertaken the collection of information on the course of illness, symptomatology, psychological testing, physical and neurological examination, cortical evoked response patterns, urinary and cerebrospinal fluid metabolites, neuroendocrine measures, and the clinical response to a single dos of dextroamphetamine. A variety of treatment approaches are being used. The research will focus on clinical and biological findings that predict response t treatment, particularly monoamine oxidase inhibitors.

(11) Neuronal and glial enolases, basic and clinical studies: Previous work b Dr. Marangos has shown that specific molecular markers can differentiate neuronal from glial elements in the central nervous system. The basic importance of these markers to neurobiology is being increasingly realized. This year ha seen a rapid growth in the number of research groups seeking collaborations fo differential cell types. The markers are two isoenzymes of enolase, referred t as neuron specific enolase (NSE) and non-neuronal enolase (NNE). NSE is prese in all neurons, and NNE is localized within glial cells in the nervous system. Major findings this year in this research are as follows:

(a) Combining the immunoassay and enzymatic assay for the enolases with immunocytochemical techniques (in collaboration with Dr. Schmechel of the LCS), we have found that during neuronal maturation NSE is acquired gradually, following a switch from NNE to NSE at a definite point in maturation.

(b) NSE has been found in various peripheral neuronendocrine cells previously categorized as APUD cells, a group of cells thought to be "neuron like" in their functional characteristics. This provides the first molecular confir tion for the APUD concept.

(c) In collaborative studies NSE has been found in cultured spinal neuro and in brain aggregate cultures. These studies have established that various culture systems are representative of nervous tissue *in vivo*, making possible their use as model systems for nervous tissue.

(d) The regional distribution of NSE and NNE have been determined in monkey and rat brain, with the distributions following that expected from the relative ratio of neuronal to glial elements.

(e) Human blood platelets have been shown to contain signigicant levels NSE, thus providing the first evidence that platelets are similar to neurons i some respect, further supporting their use as a neuronal model system.

(f) With the development of a more sensitive assay using I^{125} labelling, levels of NNE and NSE are now measurable in CSF and serum of patients. A variety of neurological and neuropsychiatric illnesses are being studied. Patients with stroke show elevations of NSE at various times following the stroke, and this may represent a marker for the course of the tissue destruction process. High NSE levels in amniotic fluid of pregnant women have been shown to correlate with some neurological defects in the fetus. These preliminary studies suggest the possible diagnostic use of these molecular markers in some clinical conditions.

The importance of the enolase markers has been recognized by the fact that the New England Nuclear corporation has approached the Branch regarding the manufacture of an RIA kit for the enolases, as well as marketing of the antisera for direct immunocytochemical studies.

(12) CNS benzodiazepine receptors: physiological and pharmacological studies: The identification of saturable high affinity and stereospecific binding sites for benzodiazepines in mammalian central nervous system has stimulated considerable interest. Our laboratory has undertaken a series of pharmacological and physiological studies of these receptors, particularly employing novel *in vivo* techniques. Major findings in these studies this year are as follows:

(a) An *in vivo* method for labelling these receptors has been accomplished; regional and subcellular distribution studies indicate a very high rank order correlation between *in vivo* and *in vitro* binding.

(b) Benzodiazepine receptors were found to be important in the mediation of metrazol-induced seizures in mice. A high correlation was observed between the number of diazepam-occupied receptors and the degree of protection against seizures. In the first study directly relating occupation of central receptor sites with the clinical effects of a drug, it was found that no more than 30% of the receptors need to be occupied for full anti-convulsant protection. Following convulsions induced chemically or electrically, there was a rapid increase in the number, but not the affinity, of benzodiazepine receptors, that lasted for less than one hour.

(c) A simple and sensitive radio receptor assay for total benzodiazepines in plasma and other biological tissues was developed. It was shown that the sensitivity of this very simple assay was equal to or greater than the cumbersome GC-MS methods.

(d) The receptor has been located in retina, in primary spinal cord cultures, and various brain tissue culture models.

(e) An irreversible, non-competitive inhibitor of benzodiazepine binding (irazepine) was developed and tested.

(f) Significant reductions in benzodiazepine receptor number were found in animals undergoing alcohol-induced seizures.

(g) Differences in benzodiazepine receptors were found in genetically inbred "nervous" mice compared with controls.

(13) Endogenous ligands for the brain benzodiazepine receptor: The discovery and further characterization of the brain benzodizepine receptor (see above) stimulated our attempts to identify endogenous ligands for this receptor, or in other words, endogenous "benzodiazepine-like" compounds. Major results of these studies are as follows:

(a) The initial identification of peaks of activity on various chromatographic separation systems has been extended, and two substances have been identified as specific ligands for the benzodiazepine receptor. These substances have been identified as the purines, inosine and hypoxanthine. A broad spectrum of naturally occurring and synthetic purines have been shown to inhibi diazepam binding, and structure activity relationships have been established. Purines are specific inhibitors of diazepam binding, and have no effect on othe receptor ligand systems, such as the opiate, the adrenergic, and the cholinergi receptors.

(b) The common methylxanthine stimulants, caffeine, theophylline, and theobromine (methylated purines) were found to inhibit competitively diazepam binding; the relative potencies in the binding assay are directly parallel the relative stimulant potencies.

(c) Direct intracerebral administration of inosine more than doubles PTZ-induced seizure latency, i.e., a benzodiazepine-like effect.

(d) Direct iontophoretic application of inosine onto cultured mouse spina cord neurons activates an inhibitory and an excitatory conductance mechanism in a way similar to fluorazepam, a diazepam-like compound.

(e) Work is continuing on the identification of other non-purine compound that may bind to this receptor.

(14) Endocrine effects on CNS neurotransmitters and receptors: These studies under Dr. Paul's direction, were initiated in the Laboratory of Clinical Scienc and have been continued since Dr. Paul joined the Clinical Psychobiology Branch

The major finding this year has been that estrogen compounds in hypothalamic preparations result in significantly increased norepinephrine efflux. Ther is a marked correlation between the concentration of estrogen receptors and thi release phenomenon when studied regionally. This suggests that the catecholamine efflux observed may be related to an estrogen receptor interaction.

Annual Report of the Laboratory of Clinical Science
National Institute of Mental Health
Irwin J. Kopin, M.D., Chief,
October 1, 1978 through September 30, 1979

Introduction

The research programs of the Laboratory of Clinical Science are concerned with a broad area of Neuroscience directed at elucidating the fundamental processes which govern neuronal development and function and applying these concepts to clinical investigations of the biological bases for disorders of the nervous system and the rational pharmacological treatments of neuropsychiatric disorders. Within the Laboratory there are four Sections (Pharmacology, Histopharmacology, Medicine, and Experimental Therapeutics) and three Units (Clinical Biochemistry, Pharmacological Applications of Mass Spectrometry, and Neuroendocrinology). The organization and direction of the Laboratory is aimed at providing an environment in which the creativity, dedication, and resourcefulness of individual scientists can best flourish. An important aspect of the program is to ensure interactions which will bring rapid application of advances in basic information or of newly developed techniques to clinical investigations and to encourage clinical problems to stimulate and influence the direction of fundamental research.

The various sections in the Laboratory each have independent research programs but common interests; the common interests in fundamental processes frequently lead to collaborative efforts with other laboratories at NIMH or NIH as well as among the various administrative divisions of the Laboratory. The Units are involved in both independent research and in providing support to the clinical research program. These interactions of the various divisions of the Laboratory are reflected in the descriptions of their research.

Section on Pharmacology
Julius Axelrod, Ph.D., Chief

The Section on Pharmacology has continued its several areas of research on fundamental processes which control neurotransmitter release, membrane biochemistry and its relation to receptors, circadian rhythms, and neurotransmitter abnormalities in experimental hypertension.

Work done during the past year has shown that phospholipid methylation influences many membrane events (Hirata, Strittmatter, Axelrod, and Crews). Stimulation of β-adrenergic receptors in rat reticulocytes increases phospholipid methylation, membrane fluidity, and coupling of the β-adrenergic receptor and adenylate cyclase. β-Adrenergic agonists stimulate methylation in HeLa cells and astrocytomas. Benzodiazapam, an anti-anxiety drug, also stimulates phospholipid methylation in astrocytoma cells. Experiments indicate that phospholipid methylation can occur on separate domains on the membrane. Rapid desensitization of β-adrenergic receptors is prevented by inhibiting phospholipase A_2, an enzyme that metabolizes methylated phospholipids. Ca^{+2} ATPase activity is elevated after methylation of membrane phospholipids is increased.

Lectins such as concanavalin A (Con A) interact with cell surface receptors and release histamine from mast cells. The lectin also stimulates phospholipid methylation and degradation of methylated phospholipids. A close association between phospholipid methylation and histamine release was found. Con A also stimulates conversion of phosphatidylserine to methylated phospholipids. Mitogenesis in rat lymphocytes has been found to be associated with phospholipid methylation. A close correlation between Con A-evoked mitogenesis, phospholipid methylation and arachidonic acid liberation was observed. Peptides that cause chemotaxis in rabbit leukocytes increase turnover of methylated phospholipids. The rapid turnover of methylated phospholipids by chemoattractant peptides leads to an increased release of arachidonic acid, the precursor of prostaglandins.

Two enzymes have been found in brain that convert phosphatidyl ethanolamine to phosphatidyl choline. These enzymes are highly localized in nerve terminals.

Studies on the pineal gland have been continued by Dr. Zatz. The circadian rhythms in rat pineal serotonin N-acetyltransferase activity can be modified by drugs acting on the central nervous system. The acute effects of light can be mimicked by several drugs injected into the brain. Of these, only carbachol is able to cause a phase shift in the temporal pattern of enzyme activity, indicating an effect on the mechanism generating circadian rhythmicity. The acute effect of carbachol appears to be mediated by a nicotinic receptor. It is not blocked by muscarinic blockers but can be blocked by tubocurarine or α-bungarotoxin. Bungarotoxin is able to block the acute effect of light on the rat pineal.

Intraventricular carbachol also has prominent effects on behavioral activity rhythms in rat and hamster. Chick pineals show a circadian rhythm in indoleamine metabolism similar to that of rats. However, unlike rats, their rhythm is not driven by adrenergic stimulation or increased cyclic nucleotides. The chick pineal displays endogenous "timekeeping," and perhaps rhythmicity, in explant culture. It is also directly photosensitive.

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Dr. Saavedra continues his studies on the role of the central nervous system in experimental hypertension. Changes in brain adrenaline and noradrenaline occur in all forms of experimental hypertension studied (DOCA-salt, neurogenic, spontaneous and sodium dependent). Increased synthesis of adrenomedullary catecholamines is present in all types of hypertension. Acute and chronic stress also result in similar changes in brain adrenaline metabolism.

Sodium dependent hypertensive rats (Dahl rats) show specific changes in brain catecholamines. Some of these changes are related to the hypertension and some to the ingestion of a high sodium diet. Genetic spontaneously hypertensive rats present changes in vasopressin and somatostatin levels in brain under normal conditions and after acute stress. Rats with diabetes insipidus (Brattleboro rats) show localized and specific changes in brain catecholamine metabolism.

These findings indicate that brain and adrenomedullary catecholamines are involved in the development and maintenance of at least four types of hypertension, and in the stress reaction. Localized catecholaminergic neurons may be involved in the central regulation of water and sodium intake. Brain vasopressin and somatostatin are involved in genetic hypertension and in the central stress response, possibly in connection with central catecholamines.

Dr. Hoffman is examining the metabolism of catechol estrogens. In experiments designed to characterize the enzyme that synthesizes catechol estrogen from estrogen it was shown that the enzyme is not induced by phenobarbital or 3-methylcholanthrene. Brain and liver enzyme activities fall after castration or prolonged hypothyroidism. Nearly all rat tissues studied have catechol estrogen synthesizing activity, but male tissues have higher specific activities than do female. Human breast tumors, but not normal breast tissue, also have catechol estrogen-forming activity and preliminary studies suggest that catechol estrogens stimulate cultured human breast tumor cells.

Catechol estrogen synthesis also occurs in liver via enzymatic demethylation of 2-methoxyestrogens. A rapid, sensitive and specific assay for this demethylase was devised. The enzyme(s) responsible for this reaction is a cytochrome P450 which can be induced by phenobarbital but not by 3-methylcolanthrene. No detectable demethylation occurs in brain, however.

The cytochrome P450 enzyme system in brain may play a significant role in the local control of CNS drug concentrations. It was shown that p-hydroxyamphetamine is converted to a catecholamine by this brain hydroxylase. Phenolic narcotics, however, do not appear to be converted to their catechol metabolites in brain. Naturally occurring amines, tyramine and octopamine, are substrates for this enzyme. Their hydroxylation represents an alternative pathway for catecholamine synthesis in brain.

Section on Histopharmacology
David M. Jacobowitz, Ph.D., Chief

This Section has previously been involved with studies of monoaminergic and cholinergic neurons in the central nervous system. More recently immunocytochemical and neurochemical localization of peptide-containing nerves in brain and histochemical and biochemical analyses at the level of discrete central nuclei and pathways have been performed to define the biochemical processes which may provide a basis for behavioral changes.

Using an immunocytochemical approach, an extensive system of neurons containing α-melanocyte stimulating hormone (α-MSH) was identified and mapped in the rat brain. α-MSH concentrations throughout the rat brain were determined by combining microdissection techniques with a sensitive radioimmunoassay. The immunoreactive compound in brain was characterized by high pressure liquid chromatography combined with radioimmunoassay. The major immunoreactive component has an identical retention time as standard α-MSH, suggesting chemical identity of the peptides. Lesion of the arcuate nucleus, the site of α-MSH containing perikarya, depleted α-MSH throughout the brain indicating that these cells are the source of neuronal α-MSH in the brain. α-MSH concentrations in the pineal gland and several brain regions investigated demonstrated a marked circadian rhythm with highest concentration during the early morning. α-MSH has also been identified in post mortem specimens of human brain where there is a regional distribution of the peptide which is quantitatively and qualitatively similar to that found in rat and cat brain. α-MSH immunoreactivity has also been identified in rat and human cerebrospinal fluid (CSF). Results suggest that α-MSH concentrations in CSF may provide an index for measuring α-MSH release *in vivo*. Potassium stimulated, calcium dependent, α-MSH release has been demonstrated using slices of fresh rat hypothalamus (O'Donohue, Thoa, Helke, and Charlton).

The hypothesis that substance P is the neurotransmitter of baro- and chemoreceptor nerve terminals synapsing in the nucleus tractus solitarius (NTS) was tested by measuring the content of substance P in four regions of NTS and in the sensory ganglia containing cell bodies of baro- and chemoreceptor afferents, and determining the effect of intracranial sectioning of the IX and Xth nerves on the substance P content of the four regions of the NTS. Measurement of substance P in NTS and sensory ganglia revealed a high level of this material in parts of NTS where baro- and chemoreceptor afferents synapse, and in nodose and petrosal ganglia. Denervation of cranial nerves IX and X of cats resulted in a 50% reduction of substance P content of parts of NTS where these afferents terminate, but no significant reduction in substance P content of parts of the NTS not involved in regulation of cardiovascular function (Helke, Gillis, Hamilton and Norman).

An antiserum for bombesin (a tetradecapeptide) was used to map the regional distribution of bombesin-like immunoreactivity (BLI) in the rat brain. Using immunocytochemical techniques, BLI was observed in discrete varicose nerve fibers throughout the brain. Cell bodies were observed in the preoptic, hypothalamic and mammillary nuclei and in the mesencephalon. Using a radioimmunoassay combined with microdissection methods, BLI was observed in the preoptic, hypothalamic and mammillary nuclei and in the mesencephalon. Using a radioimmunoassay combined with microdissection methods, BLI was observed to be 30-fold greater in high regions than low regions. When brain extracts were fractionated using high pressure liquid chromatography, one major peak of immunoreactivity was obtained which was distinct from substance P. Release of bombesin-like peptides from rat hypothalamic slices increased 2-3-fold when the brain slices were treated with depolarizing stimuli such as K+ or veratridine in the presence of CA++ (Moody, O'Donohue, and Thoa).

Immunocytochemical studies of the opiate ligand, enkephalin, revealed the distribution of a population of large axonal-like processes throughout the rat forebrain. The mapping of the distribution of these axonal-like processes, in addition to perikarya of the forebrain was undertaken. A large area of enkephalin-containing axons was observed just ventral to the globus pallidus, in the supraoptic commissure, ansa lenticularis, stria terminalis and median forebrain bundle. Enkephalin cell bodies were observed in the hypothalamus, (dorsomedial, paraventricular, periventricular, ventromedial and arcuate nuclei), preoptic area, bed nucleus of the stria terminalis, septal area, nucleus accumbens and the medial caudate nucleus. The widespread population of enkephalinergic cell bodies in the hypothalamus, preoptic and septal areas is consistent with the interpretation that enkephalin is generally contained within interneurons.

Evidence has been provided that CNS cholinergic neurons are altered in genetic hypertensive rats. The choline acetyltransferase (ChAT) activity was measured in numerous discrete brain nuclei of 4, 8, and 12-week old spontaneously hypertensive rats (SHR) and of age-matched normotensive Wistar Kyoto (WKY) rats. The acetylcholine (ACh) concentration was also measured in hindbrain nuclei of 12-week old SHR and WKY. Changes in ChAT activity of several nuclei were observed in SHR of all 3 age groups. The greatest alteration (79% increase) was found in the ChAT activity of the locus coeruleus of 12-week old SHR. A 31% increase in ACh was also detected. No differences were found in this nucleus in 4- or 8-week SHR. Other nuclei known to be involved in blood pressure control which showed differences in ChAT activity in SHR compared to WKY were the paraventricular nucleus, dorsomedial nucleus of the hypothalamus, posterior hypothalamus and the nucleus reticularis gigantocellularis. A significant increase was also found in the anterior ventral thalamus in 4- and 8-week SHR (Helke and Muth).

The involvement of central cholinergic mechanisms in the feedback actions of gonadal hormones was examined by measuring choline acetyltransferase activity (ChAT) in rats and acetylcholine (ACh) concentration in microdissected brain nuclei after castration and gonadal hormone replacement. In males, castration resulted in increased plasma luteinizing hormone (LH), as well as in a) increased ChAT in the posteromedial amygdaloid nucleus; b) increased ACh in the ventral tegmental area and rostral nucleus tractus diagonalis; and c) increased ChAT and ACh in the medial preoptic nucleus. Testosterone attenuated all of these responses to castration, and also lowered ChAT in the rostral nucleus tractus diagonalis. Treatment of ovariectomized, estrogen-primed females with progesterone resulted in a surge in plasma LH, as well as in a) increased ChAT in the supraoptic nucleus; b) decreased ChAT in the caudal nucleus tractus diagonalis; c) decreased ACh in the ventral tegmental area; and d) decreased ChAT and ACh in the periventricular nucleus. The results demonstrate that cholinergic activity in several discrete brain regions known to be targets for testicular and ovarian hormones is altered by gonadectomy and gonadal hormone treatment. These brain areas may represent sites where circulating gonadal steroids initiate neuroendocrine feedback actions through cholinergic mediators (Muth and Crowley).

Levels of serotonin were measured in microdissected, individual brain nuclei in ovariectomized rats after treatment with ovarian hormones. Regions sampled included nuclei in the forebrain, rostral and medial hypothalamus, and midbrain tegmentum. Estradiol benzoate decreased levels of luteinizing hormone in plasma but did not affect serotonin levels in any region. Progesterone alone elevated serotonin content in the nucleus tractus diagonalis and ventral tegmental area. The combined estrogen plus progesterone regime produced a surge in plasma luteinizing hormone and also markedly elevated serotonin in the median eminence. These results are relevant to the neural control of gonadotropin secretion, ovulation and mating behavior (Crowley, O'Donohue, and Muth).

An attempt was made to correlate genetic aggressivity with catecholamine dynamics in discrete brain regions of mice. It was noted that some strains of mice, when placed in isolation, become aggressive while others do not (non-aggressive). The norepinephrine steady-state level was significantly lower in the olfactory tubercle and substantia nigra and significantly higher in the septal area of the aggressive mice. Norepinephrine turnover was only higher in the A-10 region (ventral tegmental area) of the aggressors. Dopamine steady-state level and turnover was lower in olfactory tubercle and higher in caudate putamen of the aggressors. The changes in the steady-state level and turnover of DA in the caudate putamen and olfactory tubercle suggests the involvement of DA in the motor function associated with isolation-induced aggression, as well as the involvement of the olfactory mechanism in aggressive behavior (Tizabi and Massari).

We have demonstrated previously that the nucleus tractus diagonalis exhibits cyclic changes in dopamine content over the estrus cycle, changes in dopamine turnover after ovarian hormones, and sex differences in dopamine that are apparently under the control of neonatal androgen. This region appears to be a site for complex interactions among several neurochemical systems in response to gonadal steroids. Neurochemical studies were carried out to determine the site of the dopamine cell body localization which projects to the nucleus of the tractus diagonalis. Stereotaxic lesion of the A-10 dopamine cell bodies resulted in an 84% decrease in the dopamine content of the nucleus tractus diagonalis thereby revealing the site of the dopaminergic input to this nucleus (Muth).

Section on Experimental Therapeutics
Michael H. Ebert, M.D., Chief

The Section on Experimental Therapeutics has continued to study a variety of neuropsychiatric disorders, including Altzheimer's dementia, Gilles de la Tourette's syndrome, hyperactive syndrome of childhood, anorexia nervosa, and chronic orthostatic hypotension resulting from autonomic nervous system dysfunction or other causes. Studies of catecholamine metabolism in vivo in primates and in man have complemented these studies.

Work has continued on the development and evaluation of a technique to estimate the rate of homovanillic acid (HVA) production in human brain using information about the kinetics of HVA elimination and distribution both before and after administration of probenecid. This was examined in detail in the rhesus monkey (macaca mulatta). HVA labelled with two deuterium atoms (d_2-HVA) was used to label the peripheral body pool of endogenous HVA (d_0-HVA). After rapid intravenous injection of d_2-HVA, the concentrations of both d_2- and d_0-HVA were determined by gas chromatography-mass spectrometry in sequential samples of serum and urine. The decline of serum d_2-HVA concentration with time was multiexponential, with a biological half-life ranging from 35.9 to 102 min. In most animals, only about 50% of the administered dose of d_2-HVA was recovered unchanged in the urine, and renal clearance was 3.79-17.0 ml/kg/min. The calculated basal rate of HVA production by the whole body was 5.23-12.6 μg/kg/hr. During probenecid infusion, the apparent rate of HVA production by the body was increased. This was demonstrated to be due to a decrease in the apparent volume of distribution of HVA after probenecid. Preliminary results, after considering the change in volume of distribution indicate that approximately 70 percent of the total body HVA produced in the rhesus monkey is derived from the brain (Elchisak, Ebert, Polinsky, and Kopin).

Dopamine sulfate ($DASO_4$) is a metabolite of dopamine which occurs in several animal species. Work has been initiated to demonstrate the occurrence of this compound in brain fluids of primates, including humans. Methods adapted from the literature were used to synthesize and separate three different sulfate conjugates of ^{14}C-labelled dopamine. Dopamine-3-0-sulfate, dopamine-4-0-sulfate, and dopamine-3,4-disulfate. The radiolabelled standards were then used to determine optimal conditions for hydrolysis of DASO to free DA, as well as to develop procedures for the isolation of the different conjugates from body fluids. Currently, $DASO_4$ is being hydrolyzed and measured as free dopamine. A method utilizing gas chromatography-mass spectrometry has been adapted from the literature to quantitate dopamine from tissues or fluids, and modified to detect approximately 25-50 picograms of dopamine per sample. $DASO_4$ has been detected in ventricular and lumbar CSF of rhesus monkeys, as well as blood and urine of both monkeys and humans. The concentrations of $DASO_4$ are substantially greater than those of free dopamine in all these fluids (Elchisak and Ebert).

Pharmacological studies of memory function have continued this year in collaboration with Drs. Weingartner, Sitaram and Gillin. Parameters of memory processing and retrieval that were previously documented in Huntington's chorea are now being studied in a series of patients with Altzheimer's dementia and Korsakoff's psychosis. When concluded, these studies will indicate the specificity of the processing and retrieval changes that we have documented in these several dementias. A clinical trial of acute administration of arecholine (a cholinergic receptor agonist), lecithin (a dietary source of choline), and amphetamine in Altzheimer's dementia and Korsakoff's psychosis is underway. Clinical neurochemical studies of norepinephrine, dopamine, and serotonin metabolism are also underway in these dementias (Kaye and Ebert).

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The clinical research in primary anorexia nervosa has continued (Kaye, Gross, and Ebert). With conclusion of the clinical trials of lithium carbonate and delta-9-tetrahydrocannabinol a variety of neuroendocrine and neurochemical studies are in progress to further delineate the pathophysiology of the anorexia nervosa syndrome. Lithium carbonate was studied in a prospective, double blind, parallel design trial of 8 lithium and 8 placebo treated patients. The trial lasted 4 weeks and both groups received behavior modification treatment. There was a statistically significant gain of weight over time in both groups, but the effect of lithium was not significant. With regard to psychometrics, a number of anorexic behaviors and mood items improved over time significantly, but there was no evidence of a significant drug effect. In collaboration with Drs. Wachslicht-Rodbard and Roth, a study of insulin receptors in anorexia nervosa has been completed. Untreated patients showed increased insulin binding to receptors on erythrocytes (mean ± S.E.M., 12.2 ± .99 percent of total); after weight gain, these increased levels returned to normal (6.8 ± .42 vs. 6.7 ± .63 percent of total). Increased binding was due to an increased number of receptors per cell, with little or no change in receptor affinity. In 5 patients, results of insulin binding to erythrocytes correlated closely with results obtained with monocytes, suggesting that these insulin receptor changes are taking place in other tissues as well. Several neuroendocrine studies are in progress to further delineate the hypothalamic dysfunction that occurs in acute anorexia nervosa. These include CSF determination and plasma vasopressin levels after hypertonic saline infusion, plasma and urinary melatonin rhythm, and prolactin and luteinizing hormone responses after dopamine infusion (Gold and Lewy). Initial data from intravenous probenecid tests performed in patients while they were acutely ill and after substantial weight gain indicate decreased serotonin and dopamine synthesis in the central nervous system when the patients are ill.

In Gilles de la Tourette's syndrome two therapeutic drug trials have been concluded, a therapeutic trial of lecithin is beginning, and family studies continue (Polinsky, Nee, Caine, and Ebert). Among the first 50 patients studied, 16 patients had a family history for tics. Twenty-four families had more than two members with Tourette's syndrome or tics. Among the 50 patients there was a high frequency of sleep disturbance, learning disability, self-destructive behavior, inappropriate sexual activity, and antisocial behavior. Family history was significantly related to the occurrence of sleep disturbance, obsessive-compulsive behavior, and positive haloperidol response. The precise mode of genetic transmission in familial Tourette's syndrome remains to be determined. A double blind, placebo controlled trial of clozapine (a neuroleptic which has not been reported to cause tardive dyskinesia) showed that this drug was not efficacious in the treatment of Tourette's syndrome. In a double blind crossover trial, chlorimipramine and desmethylimipramine in Tourette's syndrome were also shown to be ineffective as a treatment for this disorder.

Twenty patients with orthostatic hypotension have been studied (Polinsky, Ebert, and Kopin). These patients fall into three clinical categories: (1) predominantly male, middle-aged and with significant autonomic deficits, as well as evidence for widespread central nervous system disease (Shy-Drager Syndrome); (2) equal sex distribution with wider age range, somewhat less severe autonomic dysfunction and no evidence of central neurological disorder (Idiopathic Orthostatic Hypotension); and, (3) young females with orthostatic hypotension and tachycardia without evidence of autonomic dysfunction or other neurological signs. A study of the pressor and catecholamine responses to intravenous norepinephrine, angiotensin and tyramine has been completed. In normal subjects, the mean blood pressure is proportional to the log of the plasma norepinephrine level which is related to the rate of its infusion. Similarly, increases in mean blood pressure produced by angiotensin II appear to be directly proportional to the log of the rate of infusion. In normals, bolus injections of tyramine elicit no significant increase in blood pressure until the dose exceeds 30 µg/kg. Patients with Shy-Drager Syndrome have normal basal plasma norepinephrine levels which do not change appropriately in response to postural change. These patients are also supersensitive to norepinephrine and angiotensin, but not to tyramine. These data suggest that the distal sympathetic nervous system is relatively intact, but is not appropriately activated by centrally mediated reflexes. Patients with idiopathic orthostatic hypotension have low basal plasma norepinephrine levels which change little in response to standing. This group is extremely supersensitive to norepinephrine, angiotensin, and tyramine. The supersensitive responses indicate that the effector organs (arterioles) are capable of contraction, that the sympathetic nerves are present and contain stores of norepinephrine which can be released by tyramine but that the level of sympathetic activity is abnormally low. This is analagous to sympathetic ganglionic blockade. Patients with orthostatic tachycardia have normal basal levels of plasma norepinephrine which show an exaggerated response to standing and appear to be subsensitive to norepinephrine. The defect in this group must therefore, be post junctional (distal to the sympathetic nerve endings), possibly involving the receptor or effector mechanism.

An examination of the catecholamine response to insulin-induced hypoglycemia has also been completed in the orthostatic hypotension patients. After the lowest level is reached, the rate of increase appears to be the result of two factors in blood glucose. There is an initial phase with a steep rise of glucose lasting about 20-30 minutes ("fast phase") followed by a more gradual increase, until normal levels are attained ("slow phase"). The log of the plasma epinephrine concentration during the rapid phase (area under the curve in pg/ml/min) is proportional to the slope of the initial "fast phase" and also to the difference in slopes between the fast and slow phases. A number of patients have been identified who show no increases in plasma epinephrine during hypoglycemia; these patients did not exhibit a "fast phase" in their glucose recovery curves. Despite an absent epinephrine response, these patients did have intact glucagon, growth hormone, and cortisol responses, suggesting that these hormonal mechanisms are independent, at least in part, of the epinephrine response and that the recovery from hypoglycemia is not dependent on epinephrine.

Section on Medicine
Irwin J. Kopin, M.D., Chief

The central nervous system mediates compensatory physiological responses to environmental stimuli, stress, and alterations in body temperature, O_2 and CO_2 content of blood, blood glucose levels, etc. The magnitude of the responses are influenced by the intensity of the afferent stimulation which reaches the central nervous system (CNS), the responsivity of the appropriate nuclei in the CNS, and the sensitivity and capacity of the effector organ to respond to the neurohumors released from peripheral nerve endings. Plasma levels of catecholamines and the alterations in those levels which normally attend postural change, physical exertion, or stress are valid indices of sympatho-adrenal medullary activity in patients as well as in experimental animals. The responses to administered pressor agents, particularly in animals in which the compensatory reflexes have been prevented, either pharmacologically or by destruction of the central nervous system, allows assessment of effector organ responsivities separately from neuronal influences.

Plasma norepinephrine levels are directly proportional to sympathetic outflow from the spinal cords of pithed rats, but the blood pressure elevation is proportional to the logarithm of the increases in plasma norepinephrine (Yamaguchi and Kopin). The norepinephrine and epinephrine derived from the adrenal medulla are uneffective in elevating blood pressure; the pressor responses to sympatho-adrenal medullary stimulation are the sole result of release of norepinephrine from sympathetic nerve endings.

Drugs which block reuptake of norepinephrine enhance the pressor response to sympathetic stimulation and result in greater increases in plasma norepinephrine while diminishing the amounts of 3,4, dihydroxyphenyl glycol and its metabolites released into the circulation (Jimerson, Yamaguchi, and Kopin). This confirms the view that deamination is an important means of metabolic inactivation of norepinephrine taken up into the nerve endings. A series of studies based on measurement of plasma catecholamines and the corresponding variations in heart rate and blood pressure have been in progress to assess genetic factors, pharmacological effects, central nervous system control and clinical disorders with regard to the relationship between cardiovascular or metabolic changes and sympatho-adrenal medullary activation.

Sympatho-adrenal medullary activation is an integral part of the response to stress. Pharmacological studies using drugs which do not enter the brain but which block ganglionic transmission (hexamethonium) or release of NE from sympathetic nerve endings (bretylium) suggest that the behavioral responses to stress are modulated by a positive-feedback influence dependent on sympathetic neuronal, but not adrenal medullary, release of catecholamines (McCarty and Kopin). This may be related to the good correlation between increases in plasma levels of catecholamines and behavioral activation which was found when severa different strains of rats were studied (McCarty and Kopin). The rats which were most behaviorally activated by exposure to stres were spontaneously hypertensive (SHR) rats. The most reactive of the normotensive strains of animals were Wistar-Kyoto (WKY) rats, the strain from which SHR rats were developed. During exposure to stress or in anticipation of stress, behavioral activation, outflow of sympathetic impulses from the central nervous system, increases in plasma levels of norepinephrine and elevation of blood pressure and heart rate are greater in SHR than in the WKY rats (McCarty, Kvetnansky, Thoa, and Kopin).

To separate the effects of stimulation of peripheral sympathetic nerves from those of stress-induced responses which are mediated by the central nervous system, pithed rats were used. In this preparation the effects of direct electrical stimulation of the sympathetic outflow on blood pressure and plasma catecholamines could be examined (Yamaguchi and Kopin). The results of this study showed that WKY and SHR rats released the same amounts of catecholamines into the blood during sympathetic stimulation but that the blood pressure responses were greater in SHR rats. Examination of the effects of intravenousl administered catecholamines confirmed the conclusion that the pressor effects of norepinephrine and epinephrine are greater in SHR than WKY rats. Thus two distinctly separate abnormalities appear to contribute to the development of this animal model of human essential hypertension. In the hypertensive animals, there is a greater central nervous system activation of the sympathetic nervous system *and* a greater peripheral responsivity to the released neurotransmitter. They may also be diminished vasodilator responses to isoproterenol (β-adrenoceptor subsensitivity in arterioles) and deficits in baroreceptor function in awake, intact SHR rats (Weinstock, McCarty, Zavadil, and Kopin).

In patients receiving diuretics for treatment of hypertension there are increases in plasma levels of norepinephrine, presumably as a consequence of baroreceptor activation secondary to the decline in an established elevation in blood pressure. This compensatory mechanism may be of importance in patients with resistance to diuretic treatment and addition of a drug (e.g., clonidine) which diminishes sympathetic activity provides a rational way of obtaining a therapeutic response in such patients (Lake, Ziegler, Coleman, and Kopin).

During development of the cerebellum of rats enzymes which control synthesis of neurotransmitters appear and increase in concentration at different times, while enzymes which control polyamine synthesis increase only transiently and apparently are involved in triggering different phases of development (Gilad and Kopin). After axonal injury, changes in enzyme levels result in a pattern similar to that seen during development.

Using immunohistological techniques enolase characteristic of nerve cells have been found in peptide secreting cells in midline organs of the brain in α_1-thymosin-containing cells of the medullary centers of the thymus, and in other peripheral endocrine cells. The ontogenetic relationship between such cells and nerve tissue is being examined (Schmechel, Oertel, and Marangos).

A radioactively labelled irreversible inhibitor of glutamic acid decarboxylase (GAD) was successfully used to localize the enzyme on two-dimensional electrophoresis. Antibodies to the enzyme have been produced and have been used to demonstrate the presence of GAD in specific cells in the nervous system (Oertel and Weise). Since this antibody inhibits GAD activity in parallel with cysteine sulfonic acid decarboxylase activity, it is likely that the same enzyme catalyses decarboxylation of both amino acids (Oertel and Weise).

Office of the Chief

Irwin J. Kopin, M.D., Chief

The Units in the Office of the Chief provide a source of support for clinical studies as well as conducting independent research or developing of methods which can be applied to clinical studies. There is a new Unit on Neuroendocrinology (Dr. Michael Brownstein) as well as the older Unit on Pharmacological Applications of Mass Spectroscopy (Dr. Sanford Markey) and a Unit on Clinical Biochemistry (Mrs. Edna Gordon).

Dr. Sanford Markey has extended the use of high sensitivity gas chromatography negative chemical ionization (GC-NCI) mass spectrometry to the analysis of biogenic amine metabolites. Drs. Blombery and Markey have developed hexafluoroacetone as a derivatizing reagent for 1,2 diols, amino alcohols, and hydroxy acids by GC-NCI. With this derivatization reagent, the detection limits for the norepinephrine metabolites MHPG, DHPG, normetanephrine, and metanephrine have been improved so that assays of these compounds can readily be performed on 0.5-1.0 ml of human plasma.

Drs. Tetsuo and Markey have developed a GC-NCI assay for urinary 6-hydroxymelatonin sulfate, the major melatonin metabolit based on a stable derivative of 6-hydroxymelatonin developed by M. Sisak and S. Markey. Studies are in progress to determine levels in normal men during various times of the day and night to correlate plasma melatonin levels with urinary 6-hydroxymelatonin excretion and to determine metabolite levels in severa experimental animal studies (pinealectomized and intact animals of several species).

Dr. Alfred Lewy has continued to develop and apply a highly sensitive GC-NCI technique for measurement of plasma levels of melatonin. Pinealectomized rats have almost no circulating melatonin indicating that this compound is from the pineal only and not from any of the hypothesized extra-pineal sites. Studies of drug effects on plasma melatonin levels are being tested as a means of determining pineal β-adrenoceptor sensitivit in humans *in vivo*.

Dr. Colburn has used GC-NCI for measurement of serotonin. Increased sensitivity has permitted the detection and quantification of serotonin in human cerebrospinal fluid. It is also possible to estimate the rate of conversion of orally administered deuterated tryptophan to deuterated serotonin in rat platelets, brain, and gut. These studies are being pursued to permit synthesis rates of serotonin to be assessed in man.

Enhanced sensitivity and semi-automated operation have also resulted from the completed SIRS (Selected Ion Recording System) data system developed by DCRT in collaboration with Dr. Markey and Mr. Oliver. The system has been expanded and now operates well with quadrupole as well as magnetic sector mass spectrometer

Drs. Richard Hawks and Stanley Burns (NIGMS) applied GC-MS methods for measurement of phencyclidine (PCP) to generate information on the pharmacokinetics of PCP after its acute and chronic administration to rats and monkeys. Preliminary studies on the excretion rate of PCP versus plasma pH were carried out to test the validity of acidification as a means of overdose treatment. These experiments continue this summer. Other studies are designed to investigate the role of PCP metabolites in the chronic effects of PCP. A deuterated standard for hydroxy-PCP metabolite is being prepared by NIDA contract and will be used as an internal standard for a more sensitive GC-MS assay.

The Unit on Clinical Biochemistry under Edna Gordon develops and applies new procedures for the quantitative analysis of biogenic amines and their metabolites in animal and human tissues and in biological fluids. A number of techniques are employed but since stable isotopes are so often used for clinical studies, gas chromatography-mass spectrometry (GLC-MS) is most often the appropriate method for final measurements. The GLC-MS is most often the appropriate method for final measurements. The GLC-MS also provides increased sensitivity and specificity of metabolites present in plasma and cerebrospinal fluid. Analysis requires preliminary purification and concentration by ion exchange chromatography and high pressure liquid chromatography (HPLC) and lyophilization of the samples.

The main project in progress at present is the study of turnover of norepinephrine in the central nervous system using ^{14}C dopamine infusion (Drs. Kopin, Ebert, and Polinsky). Methods of purification and quantification have been refined further and a number of studies in normal subjects and those with central or peripheral hypotension have been completed (Gordon and Sherman). Patients with peripheral hypotension have been found to produce less MHPG from peripheral sources than do patients with central hypotension. In both groups the brain appears to produce similar amounts of MHPG. These results show promise for studies with other patients believed to have abnormalities of central or peripheral norepinephrine metabolism.

Since the initial purification and separation of metabolites labelled with radioactivity for this study is so time consuming, dopamine labelled with ^{18}O (Oliver and Markey) is being prepared. The use of this compound instead of radioactive ^{14}C dopamine will make easier, by the use of GLC-MS, direct comparisons of ^{18}O-containing and non-labelled metabolites. To study arterial/venous differences in blood perfusing brain, very sensitive accurate and specific methods are required to measure very small amounts of catecholamine metabolites in plasma. For this purpose Drs. Blombery, Kopin, and Markey have developed GLC-MS negative ion detector analytical procedures to measure MHPG, DHPG, normetanephrine and metanephrine in plasma. These studies are in progress and appear to yield valid results.

Work with investigators outside LCS is continuing. With Drs. Goodwin, Post, Bunney, and van Kammen, Edna Gordon and Robert Sherman are analyzing cerebrospinal fluid for 5HIAA, HVA, and MHPG. Cerebrospinal fluid has been collected from a large number of normal subjects as well as from depressed or schizophrenic patients. The analyses have been completed and await correlation of biochemical and behavioral data.

In the Unit on Neuroendocrinology, Drs. Brownstein and Gainer have shown that neurons of the hypothalamo-neurohypophyseal system manufacture two 20,000 MW neurophysin procursors. One of these is not made by Brattleboro rats (animals with hereditary diabetes insipidus) and is presumed to be the precursor of the vasopressin-associated neurophysin. This precursor has now been shown to contain both vasopressin and its neurophysin; similarly the second precursor mentioned above is a common precursor for oxytocin and its neurophysin. The vasopressin-neurophysin precursor (pro-pressophysin) is a glycoprotein; the oxytocin-neurophysin precursor (pro-oxyphysin) is not. The reason for this difference is unknown but is under investigation.

Dr. Brownstein and Dr. Roth's group have shown insulin is present in the brain of the rat. Brain insulin levels do not parallel peripheral levels, and up- or down-regulation of peripheral insulin receptors is not accompanied by changes in central receptors. Insulin is also found in (and manufactured by) a number of extrapancreatic tissues besides brain. Thus, insulin may have an important and rather general role inside of cells.

The distribution of α-MSH in the rat brain has been determined by Drs. Eskay and Brownstein. Several limbic structures have especially large amounts of this peptide. The primary source of the α-MSH in the brain was shown to be neurons in the arcuate nucleus. Monosodium glutamate, a commonly used food additive, is known to be toxic to arcuate neurons; administration of this salt to neonatal animals resulted in the loss of 90% of the α-MSH from the central nervous system. Chemical characterization of the melanotropic peptides in the brain is under way. It is evident that there are melanotropic substances in addition to α-MSH in the nervous system.

The release of α-MSH from dispersed cells of the intermediate lobe of the pituitary has been examined. This _in vitro_ system has already proven to be very useful for studying the pharmacology of noradrenaline and dopamine. Isoproterenol enhances the release of α-MSH, whereas, dopamine or dopamine agonists inhibit its release. The isoproterenol-stimulated release of α-MSH seems to be mediated by an increase in intracellular c-AMP; the action of dopamine is independent of changes in c-AMP levels.

Somatostatin, the growth hormone release-inhibiting hormone is found in the hypothalamus, in extrahypothalamic brain regions and in the periphery. We have now shown that this peotide is present in the retina as well. By using a variety of techniques we hope to determine which cells make this and other active agen in the retina.

Annual Report of the Laboratory of Developmental Psychology

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Marian Radke-Yarrow, Ph.D., Chief

Summary

Developmental psychology as a discipline claims a large share of credits for research-based knowledge regarding the changing structure of child behavior from infancy to adulthood, and for knowledge about a range of factors or conditions associated with adaptive and maladaptive child behaviors. As a discipline, Developmental Psychology is a longitudinal cut through all of psychology; its substance is the psychological processes of perceiving, thinking, learning, affective responding, and so on, -- all of these in developmental perspective. The substance of Developmental Psychology comes also from content research, the relations or associations between psychological and behavioral characteristics of children and antecedent or concomitant conditions of biology and environment. The level of maturity of the organism is an explicit consideration.

Process research, content research, and, also, methodological research are represented in the work of the Laboratory in the broad domain of social and emotional development of children. Research efforts are concentrated in three general areas: (1) normal processes in affective development and factors related to good and deviant affective adaptations; (2) stressful or handicapping conditions of biomedical or social origins which potentially interfere with children's cognitive, social, and emotional development; and, (3) childhood psychopathology -- specifically, problems of measurement and of treatment evaluation.

Normal Processes in Children's Affective Development

Compared with other aspects of psychological development, normal processes in affective development have had modest attention in systematic research. The child's experience of emotion, the expression, use, and control of emotion, and the impact on the child of emotions in his/her social environment are processes that undoubtedly are interdependent, change with development, and are influenced by constitutional and experiential factors. There are, however, few bases of research evidence to which one can turn to identify the nature of these relations and the developmental changes that take place.

The research that has been undertaken in the Laboratory focuses on emotional stimuli that impinge on the child either in isolated occurrences or in patterned events in the child's life. The purpose of the program of research by Drs. Carolyn Z. Waxler and Marian R. Yarrow has been to evaluate how children process emotional events, behave in their presence, and are influenced in a cumulative way. In the first years of this research, a method was developed and perfected which combined naturalistic and laboratory procedures, and obtained detailed and continuous data records from which could be plotted developmental transformations in children's responses to the emotions of others. In the initial project, children were studied continuously over a nine month period in the second and third years of life. A second sample was studied to verify findings and developmental processes and also to extend the research to include measures of children's cognitive capacities in relation to their responses to emotions. Dr. Michael Chapman has also been involved in this research in the past year. The high density of behavior sampl permitted identification of the progressive acquisition and transformation of social responses in very young children. Of particular interest are children's empathic reactions to others' emotions expressive of distress, and the development of patterned responding to distress. One sample of children studied in the second year of life has been followed up at six years of age, and his/her behavior at age six is compared with behavior at age two. Although there is a near absence of developmental progression in the intensity of affective response to distress in other persons, there is strong evidence of carry-over from age two to age six in the individual patterns of responding to others' distress.

In another study an extensive battery of assessments is used with school age children ages four to eleven. The interrelations of mental age, cognitive role-taking capabilities, emotional empathy, and socially responsible behavior with regard to other persons in distress are examined.

In the preceding research, developmental progression has been given major emphasis. A second set of data has been obtained in the same studies, namely, data on parental rearing practices. Child misdemeanors involving harm to persons are of special interest (Dr. Carolyn Waxler). The reciprocal influences of parent and child are given attention; however, the basic purpose is to gain understanding of the contributions of parental behavior in developing interpersonal integrity in young children.

Interaction of Biomedical and Psychosocial Variables

A considerable investment of research energies is in developing understanding of the effects of chronically stressful conditions on child development and behavior. One type of stressful condition being investigated is serious chronic illness. Because they are major causes of morbidity, mortality, and great emotional suffering, cancer and juvenile diabetes were chosen for study. States and changes in biological and psychological systems are being explored using systematic observational techniques, interviews, and standard tests. The research objectives are of two kinds: those specific to the illness and those concerned with children's capacities and strategies for coping with stress -- about which there is little systematic information.

In a completed study of children hospitalized for leukemia, Drs. Elizabeth Susman and Albert Hollenbeck found that although the child and the parent have little objective control over the disease and treatment, those patients and families who perceived some measure of control in their own hands had much better psychological adjustment to the illness and maintained an orientation toward living and toward future goals. The influences of the parent and hospital staff caregivers in moderating the effects of illness have been extensively examined in these studies, and the findings have been translated into clinical care approaches to child cancer patients.

Dr. Howard Moss's work with children with leukemia centers on the psychological effects of treatment of the disease (cranial irradiation and intrathecal chemotherapy) on the cognitive and perceptual functioning of the children. The child patients compared with their sibling controls tested lower on a battery of tests of mental abilities. The differences between patients and siblings were greater for the younger patients than for the adolescent patients. A replication study that is prospective is in progress. Here the child patients are tested prior to CNS treatment and one year after treatment to establish more definitively the degree of possible impairment in mental functioning.

In two studies of juvenile diabetes, the interaction of medical and psychosocial factors in affecting the child's well-being is being investigated. Psychosocial factors and emotional stress are thought to be influential regarding adherence to treatment regimens and also regarding complications in the course of the illness. These studies are just beginning.

Because of the clash of the specific demands imposed by the illness and the psychological characteristics of the adolescent period, teenage diabetics were chosen for study by Dr. Beatrix Hamburg. Research participants are 60 metabolically labile patients (brittles), 60 nonbrittle patients and 60 normal adolescents. Some specific areas being studied are: the effects of moderate changes in glucose and other stress-related metabolites on mood and behavior, individual differences in metabolic changes that result from situations of both emotional arousal and physical activity, and effects of the psychological meaning of the disease on the health and social functioning of the adolescent and family. Emphasis is placed on classifying strategies of coping with life stress and assessing social support as a moderator of stress. This project is being carried out in collaboration with Dr. Marvin Cornblath of the Neonatal and Pediatric Medicine Branch, NICHD.

In Dr. Howard Moss's study of juvenile diabetes the focus is on the onset of the disease, the development of modal patterns of responding over the first year, and factors influential in the kind of pattern that evolves. Children 4 to 7 years and 12 to 14 years, patients and healthy controls, are the research participants. Emphasis is on behaviors relating to daily monitoring and regulating blood sugar levels, apprehension over possible later complications, and interpersonal tensions associated with the disease. Pre-illness functioning of child and family, medical management of the situation in which the child and family are given the diagnosis, and availability of support systems are examined in relation to the psychological impact of the disease.

Also falling within the framework of research on medically handicapping conditions are a project on physical handicaps in children and two studies of infants at risk due to premature birth. In the study of physical handicaps, Dr. Pizzigati addressed a social psychological problem involving the integration of handicapped children into regular classrooms. "Mainstreaming" classrooms in public education has come about as a result of recent legislation. The kinds of relations and interactions that develop between the handicapped and nonhandicapped children were studied. Small experimental groups of elementary school age children were systematically observed. Interaction differed very little in groups with, and those without, an orthopedically handicapped child present. Presence of a handicapped child was associated with a slight tendency toward more inhibition in interaction. Experience in mainstreamed classrooms appeared to have had an influence on the nonhandicapped children; those who had been attending a mainstreamed school gave greater attention to, and exhibited more prosocial behaviors to the handicapped child than did nonmainstreamed children for whom physically handicapped children had not been present in the classroom.

The behavioral outcomes of too short or too long periods of gestation are not fully understood. Evidence from late infancy and childhood indicates that children who were not born at term are at greater than average risk for intellective and behavioral deficits. Dr. Albert Caron who has been investigating information processing in normal infants has extended his research to preterm infants. Using habituation procedures, he has compared term and preterm infants' abilities to abstract relational information from stimuli. The initial findings indicate that preterm infants are less sensitive to the configuration of stimulus elements than are full-term infants. The purpose of this work is to attempt to develop a behavioral assay of intellective deficit in early infancy.

Preterm and post-term infants are the subject of Dr. Sarah Friedman's research. Infants were studied in the perinatal period with the purpose of identifying possible precursors of behavioral deficits. The infants were of weight adequate for gestational age; they were low medical risk prematures, healthy full-terms, and post-term neonates. The infants were of the same racial and socioeconomic backgrounds. The psychological functions examined were infants' sensory processing and temperament. Preterm and full-term did not differ in responsiveness to tactile stimulation. Preterms functioned only slightly (though statistically significantly) less well than full-terms in auditory and visual modalities. Post-terms were not deficient relative to full-terms. Thus, the medically healthy not-at-term infants appear to fare well on sensory functions. On measures of temperament, however, preterms and to a lesser extent post-terms show more difficult dispositions than full-term infants. They have more labile state organization, are more irritable, and are less readily soothed. In these ways they are more difficult children to care for. Understanding of the bases of such temperament differences requires further study. However, the findings are very relevant from the viewpoint of the rearing experiences of these children. The interactive effects of child characteristics and caregiver behavior are well known. Thus, nonterm birth appears to increase the probability of infant-caregiver interactional complications.

These interactive influences in early infancy are the subject of a study by Drs. Gewirtz and Hollenbeck. As the result of recent pediatric research of Klaus and Kennell, renewed attention has been given to processes of attachment and bonding in early infancy. This work suggests effects of duration of contact between infant and mother in the first postpartum hours on the mother's attachment to the infant. Drs. Gewirtz and Hollenbeck have replicated and extended these studies. The kind and duration of mother-infant contact in the recovery room were varied with different groups of mothers. Effects of contact were found in both mother and infant behaviors in observed interaction at the end of the first postpartum month. Different patterns of maternal and infant behaviors were associated with variations in mode and duration of first-hour contact conditions. Further work, not yet analyzed, involves fathers' physical contact with C-section infants within 1½ hours of delivery, and nurses' early intensive planned contacts with infants. Fathers and nurses with and without these contacts are being observed in later interactions with the infants.

The interdependencies of environmental factors, biomedical conditions, and behavioral properties of children are the subject of two parallel projects by Drs. Marian Yarrow and David Barrett on chronic moderate protein-calorie-undernutrition and functional competencies of children. Evidence from animal data and severely malnourished children suggests that a wide range of competencies is at risk in the malnourished organism. Many children who are reared in poverty in this or other cultures are chronically undernourished at a mild or moderate level. The nature and extent of psychological and behavioral impairment in children suffering "moderate" undernutrition is not known. Children from poor urban circumstances in the United States, and children from poor rural circumstances in Guatemala are the subjects in this research. Six to eight year olds who have a history of undernutrition and those who have a history of adequate nutrition are compared. Their nutritional history and status are based on anthropometric measures, food intake measures, and biochemical measures. Sensory, motor, cognitive, and social-emotional functions for which there are theoretical or empirical reasons to indicate impairment by nutritional deficit are being measured in standard tests, observations, and field experiments. In addition, ethnographic data are obtained to assess the family and community environments in which these children live. Data collection will be completed in September 1979. Findings based on comparable or identical procedures used in two cultures and by two independent teams of field investigators should help to identify stable relations between nutritional status and function, and to form the basis for hypotheses regarding the mechanisms involved.

Interrelations of Environmental Pathology and Child Behavior

Family circumstances and relationships and the functioning of individual family members provide environments in which many children experience chronic stress. Children's capacities to deal with the difficulties encountered, their means of coping, and the cost and consequences in their development are issues investigated in a number of research projects.

Such environmental conditions are often composites of multiple problems. From the standpoint of research this presents difficulties. Sometimes isolation of the contribution of a specific variable in the complex is of crucial importance; in other instances the variables in question occur almost invariably in combination and can, therefore, be dealt with meaningfully in their composite form. One such composite of continuing social and psychological stress is found in the circumstances of children of the urban poor who know only a single parent, and that parent (the mother) is in prison. Over the past three years Drs. Velma LaPoint and Marian Yarrow have studied about 50 such families. The research began with imprisoned mothers as the major source of data regarding the fate of the mother's role and functions, and the consequences for the child of growing up in these circumstances. To these data have been added direct observations of the children, and also records of child behavior in school and home. From analyses to date, some of the sources of stress and responses to stress become evident. One set of analyses is concerned with the socialization of the child into an offender attitude or role. Out of exposures such as the following is built up a sense of self in which offender-identity is almost a part of the child's self. Children often witness mothers' offenses, apprehension, and sentencing. They are inadvertently subjected to institutional processes intended as protective of, or punitive to, the mothers (e.g., being searched, being denied contact or communication with the mother, and so on). Children are taught or learn how to manipulate the "system" for their own benefit (e.g., getting into problems is sometimes an avenue to permitted contact with the mother). The children experience social prejudice directed to their mothers and to themselves. Most of the children are "on their own" in having to cope with these problems of self-concept and self-worth.

The relationship between child and mother during the incarceration are examined in another analysis. The relationships are extremely complicated and contradictory. Some children try to "write-off" the mother as a significant person to them. Some have positive ties, with strains. Many children also carry on explicit and very hostile and punitive behaviors toward their mothers. Again, there are no societal mechanisms or efforts of intervention which might serve to moderate these feelings or behaviors.

The final phases of data collection are now underway. The comparative data are being gathered on families of similar composition and from the same neighborhoods as the sample of imprisoned mothers. This will permit comparisons of developmental problems and processes in households of poverty with and without the condition of mother's institutionalization.

Other research on stressful rearing experiences involves families in which a parent is suffering from severe psychopathology. Again the focus is on the consequences for children of chronic (problem) conditions. A study involving parents with bipolar affective illness and their young children was initiated this year, in collaboration with investigators in the Biological Psychiatric Branch and the Laboratory of Clinical Psychobiology. For this study, Dr. Waxler has adapted methods from her research with normal families, and has developed new procedures of parental reporting, home and laboratory observations and standard tests which provide assessment of affective communication between parent and child. Preliminary work has also begun on a second protocol for the study of rearing by affectively disturbed mothers and the social and emotional behavior of their children. Parent and child are observed in the laboratory, which is a home-like suite of rooms, in planned situations covering a critical profile of rearing functions (such as providing for the child's routine care, providing stimulation, responding to the child's emotions, instilling standards and values, etc.), graded developmentally. The behaviors are elicited in standard but natural circumstances sampled at closely spaced intervals over a 6- to 12-month period. The sampling is sufficiently frequent to permit the evaluation of affective interaction patterns, their stability, and their responsiveness to intervention. The mothers are drawn from clinical diagnostic categories. Mothers are reclassified in terms of specific symptomatic behaviors that express their pathology. Subgroups representing distinct behavior profiles are selected for study in terms of their presumed potential for disturbed interaction and communication with the child. Environmental (rearing) factors and genetic factors are conceptualized in many studies as interactive in their contributions to behavioral outcomes. However, the levels of conceptualization and measurement for environmental and genetic variables often differ greatly, with "environment" likely to be indexed more grossly and crudely than "genetics". In the research described, it is hoped that critical environmental dimensions can be conceptualized and measured so as to move toward more nearly equalized precision in the two sets of variables. Procedures and arrangements for samples and collaborations are being planned. Drs. Marian Yarrow, Carolyn Waxler, and Michael Chapman are the investigators in this work.

Child Psychopathology

The development of a standardized system for assessing patterns of problem behaviors among disturbed children and its utilization in studies of treatment outcome have been the focus of research by Drs. Thomas Achenbach and Craig Edelbrock. When Dr. Achenbach came to NIMH in 1975 there was no well differentiated, objective method for classifying children's adaptive and maladaptive behavior. Dr. Achenbach has developed the Child Behavior Checklist and the Child Behavior Profile. The Checklist is designed to record in a standardized format the behavioral problems and competencies of children aged 4 through 16 as reported by their parents or parent surrogates. Responses to the Checklist are scored on social competence and behavior problem scales of the Child Behavior Profile. By factor analyzing Checklists for children grouped by sex and age, it has been possible to identify syndromes that are peculiar to particular sex and age groups as well as syndromes that are fairly similar among the various groups. Separate editions of the Profile have been standardized for boys at ages 4-5 and each sex at ages 6-11 and 12-16. Each edition of the Profile comprises behavior problem and competence scales on which a child's score can be compared with scores obtained by normal children of the same sex and age. The normative data were obtained from randomly selected parents of 1400 normal children. In order to reflect clinically significant syndromes, the behavior problem scales were derived through factor analyses of Checklist data on over 2,000 disturbed children referred to mental health settings. Previous empirically-based taxonomic efforts show considerable convergence on syndromes that are common across sex and age, but the traditional practice of lumping together both sexes and diverse ages has obscured syndromes that are unique to one sex or particular developmental periods. The Checklist and Profiles are now in widespread clinical and research use.

For the past three years, Dr. Craig Edelbrock has been working with Dr. Achenbach. In this context, Dr. Edelbrock has addressed a major statistical problem in mental health research, that of translating subjects' scores on a set of single dimensions into a reliable taxonomy of individuals through the use of cluster analysis (statistical procedures to produce classifications of individuals from multivariate data sets). This statistical problem is particularly significant since decisions regarding diagnosis, treatment assignment, and delivery of services need to be based on each individual's entire pattern of scores rather than on the scores of single dimensions. Many clustering algorithms are now available, but different algorithms produce different results and some algorithms are unsuited for behavioral research. The purpose of Dr. Edelbrock's research has been to evaluate several widely used procedures of cluster analysis and to develop meaningful, reliable new procedures.

The development of the Child Behavior Checklist and the Child Behavior Profile has been an initial step in Dr. Achenbach's and Dr. Edelbrock's program of research on differential etiologies, prognoses, and responses to treatment among children who differ in behavioral pattern. Another step is to assess stabilities and changes in levels and patterns of Profiles in children followed up after treatment in outpatient psychiatric clinics. Other studies have compared the power of data from parents, teachers, clinicians, and trained observers to predict the outcome of inpatient and outpatient treatment. In order to detect interactions between the effects of child characteristics and treatment characteristics, children matched on initial Profile pattern and demographic variables are experimentally assigned to either one of two treatments in an outpatient psychiatric clinic. The results of this study should make it possible to determine whether differential outcomes for children differing in initial Profile pattern are a function primarily of the child's characteristics, the treatment received, or an interaction between the two. Drs. Achenbach and Edelbrock have also conducted epidemiological research on the distribution of adaptive and maladaptive behaviors in children.

During the past year, the scientists on the Laboratory Staff have had an active role in the scientific affairs of ADAMHA and NIH, serving on a variety of advisory committees and review committees. They have presented their research to other Laboratories, to the joint meeting of the Interagency Panels on Early Childhood Research and Development and the Interagency Panel on Research and Development on Adolescence, and to other Government agencies.

This year members of the Laboratory staff organized or contributed significantly to the organization of three conferences on specialized research areas: infants at risk, juvenile diabetes, and the criminal justice system and children. Staff members have also been participants in national conferences on topics related to their research -- affective development, nutrition and behavioral functioning, and the child in the criminal justice system. They have been invited speakers for colloquia series in the United States and Canadian universities, and have presented papers at the American Psychological Association, the Society for Research in Child Development, the Classification Society, and the Mid-Atlantic Division of the American Association for Marriage and Family Therapy. Many of the investigators serve on editorial boards or as editorial consultants for the major journals and book series of the discipline.

Although much of the research will always be off-site in whatever facility or circumstance is dictated by the research problems, the main laboratory building and the adjacent new laboratory space are being heavily used for testing, interviewing, and experimental studies. As programmatic themes have developed along the lines described, an increasing share of the research has become collaborative within the Laboratory and also with investigators in other laboratories and institutes.

Annual Report of the Laboratory of Neuropsychology

National Institute of Mental Health

H. Enger Rosvold, Ph.D., Chief

October 1, 1978 to September 30, 1979

A year ago the Laboratory of Neuropsychology was reorganized into Sections more firmly based on the area of scientific interest of the senior investigators. There was some misgiving centered around the meager supply of supporting personnel; nevertheless, the reorganization was accepted with enthusiasm and had the effect of encouraging the application of new procedures, utilization of previously untried strategies of research and brave planning for comprehensive solutions of the difficult problems. Consequently the pace of the total program quickened, resulting in a more comprehensive cluster of findings. In the summary to follow this enthusiasm can be sensed and the success of the reorganization appreciated.

Cerebral Mechanisms in Object Perception and Memory

In a series of collaborative studies with Gross at Princeton, we delineated the functional pathway through which inferior temporal (IT) neurons receive their visual information. The method entailed measuring the defects in the visual receptive fields of single IT neurons after selective cerebral lesions or commissural transections. As we had predicted from our earlier ablation studies, the subcortical pathway from superior colliculus through pulvinar to IT cortex turned out to be unimportant for the receptive field properties of IT neurons. By contrast, the cortical pathway from striate through prestriate to IT cortex proved to be essential, since interruption of this pathway by a unilateral striate removal or by section of the forebrain commissures eliminated the corresponding visual input to IT cells.

We have now demonstrated this functional pathway autoradiographically using the $[^{14}C]$ 2-deoxyglucose method developed at NIMH by Sokoloff and his group. In collaboration with Kennedy in Sokoloff's laboratory we have found by comparing a blinded and a seeing hemisphere in the same monkey that the visual system can be clearly traced from the striate through the prestriate to the inferior temporal cortex on the basis of differential hemispheric uptake of the 2-deoxyglucose during visual stimulation. The studies were carried out in awake monkeys presented with visual patterns either in a rotating drum or in a discrimination apparatus. In the latter case, the animal performed the discrimination for water reward using the hand opposite the blind hemisphere. In both situations reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate projection but throughout the entire expanse of circumstriate and inferior temporal cortex as far forward as the temporal pole. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. (Side-to-side differences were also seen subcortically in the temporal lobe as well as in collicular, pulvinar and parietal regions; these additional findings are relevant to later sections of this review.)

The results strongly support the view that the visual discrimination functions of IT cortex depend on the information it receives from the striate cortex via a relay in the prestriate. Yet there is still considerable dispute concerning this proposed relay, since even massive prestriate lesions may leave visual discrmination relatively unimpaired. To resolve this apparent discrepancy we have attempted to delineate by neuroanatomical techniques the entire striate projection area within the prestriate cortex as a preliminary to removing it in a "disconnection" experiment. One series of monkeys was prepared with unilateral lesions of lateral, posterior, or medial striate cortex, such that collectively the lesions included all of area 17 with little or no invasion of area 18; their brains were processed for terminal degeneration by the Fink-Heimer procedure. In a second series, selected striate sites were injected with tritiated amino acids, and the brains processed for autoradiography; representations of the injection sites ranged from 4° to 25° from fixation in either the upper or lower visual field. The results indicate that striate cortex projects to at least three separate and topographically organized visual areas within prestriate cortex.

The largest projection field is a circumstriate cortical belt which corresponds remarkably close to area OB of von Bonin and Bailey. It completely surrounds area 17 along with the 17-18 border except at the representation of fixation. Within this visual area, the representations of the upper and lower visual fields are entirely separate. Progression from central to far peripheral vision is represented: a) in the lower field, by a progression into the posterior bank and depth of the lunate sulcus, medially along the surface of the buried annectent gyrus into the parieto-occipital sulcus; and then rostrally along the upper lip of the calcarine fissure; b) in the upper field, by a progression into the inferior occipital sulcus, ventromedially into the occipitotemporal and collateral sulci, and then rostrally along the lower lip of the calcarine fissure. A second, smaller projection field is located in area OA along the caudal portion of the superior temporal sulcus; here, progression from central to far peripheral vision is represented by a progression down the posterior bank of the sulcus and continuing along its floor. Finally, a third, even smaller projection field, also located in area OA, begins in the anterolateral part of the annectent gyrus and extends forward to occupy the depth of the lateral bank of the intraparietal sulcus. Despite its small size, evidence of a complicated topographic organization within this field raises the possibility that it may contain more than one visuotopic map.

These three projection areas thus constitute all the possible prestriate routes through which striate output might reach inferior temporal cortex. In addition to using this information to attempt a total striate-temporal disconnection, we are trying to determine whether even small prestriate lesions might disconnect IT cortex from corresponding parts of the visual field by testing visual discriminations confined to those fields in monkeys that have been trained to maintain fixation. To monitor this fixation accurately, we have just completed the design and construction of an infrared, corneal-reflection device with the aid of the Section on Technical Development.

According to the unit recording studies nearly two-thirds of IT neurons receive information from both hemispheres (i.e., from both the left and right visual half-fields). We have therefore proposed that IT cortex plays an important role in stimulus equivalence across the visual fields. This is the phenomenon in which a stimulus is recognized as the same regardless of its retinal position, even if the different retinal loci project to different hemispheres. To test our hypothesis, we prepared monkeys with bilateral IT ablations combined with section of the optic chiasm, and trained them first with one eye and then with the other eye on a series of visual pattern discriminations. Unlike control monkeys (with either IT lesions alone or chiasm section alone), who can perform immediately with the second eye any discrimination that they have learned with the first, the experimental monkey had to learn each discrimination anew with the second eye. These results provide strong evidence that stimulus equivalence across the visual half-fields depends on the convergence of their projections onto single IT neurons. We are planning to test a further prediction from this hypothesis, namely, that IT lesions will prevent transfer of a visual pattern discrimination from one part of a visual half-field to another. The method involves training monkeys whose maintained fixation is being monitored as described earlier to discriminate a pair of patterns in one quadrant of a visual half field and then test them for transfer to the other quadrant. Transfer should occur, as before, only if IT neurons are present to mediate the spatial convergence of visual information. In a related electrophysiological experiment we will test whether single neurons are indeed activated by their preferred stimuli irrespective of stimulus position in the visual field.

This spatial convergence upon single cells provides a mechanism that is essential for discrimination learning under normal viewing conditions. It insures that the same central visual cells will be activated from one exposure of the stimulus to the next despite the fluctuations in fixation, distance, direction and angle of regard that lead to stimulation of different populations of striate and prestriate cells on different presentations. But spatial convergence alone is insufficient to insure reactivation of the same central visual cells unless it is associated with a mechanism for temporal convergence. That is, a given stimulus must excite the same visual neurons on a second occasion as on the first if there is to be any summation of the effects of experience or training. We now have evidence that the IT cortex does indeed contain a mechanism for temporal convergence, as indicated by the following experiments on visual recognition.

Trained monkeys that are shown an object once will demonstrate that they recognize it as familiar several minutes later by avoiding it in favor of another object that is completely novel. Thus, somewhere in the visual system the single presentation of a complex stimulus leaves a trace against which a subsequent stimulus presentation can be matched. If it does match, i.e., if the original neural trace is reactivated, there is immediate recognition of familiarity, and the behavioral consequence described above ensues. The area in which the neural trace is first established turns out to be the anterior IT cortex, since removals here but not elsewhere abolish the animal's ability to recognize an object that it has seen once just a few seconds before. Apparently, the anterior IT cortex contains the traces laid down by previous viewing, and these serve as central representations against which incoming stimuli are constantly being compared. In the process, old traces may either decay, be renewed, or even refined, and new traces are added to the store. An experiment is now being designed to study these newly localized visual traces at the single-unit level. The question to be addressed is whether or not a neuron that at first responds equally to two similar stimuli can be induced to respond differently to them by training the animal to attend to their physical differences.

The evidence that has been cited favors a sequential activation model for object vision. Information that reaches the striate cortex is transmitted for further processing to the prestriate cortex, and from there to the IT area. Can this model be extended beyond vision per se to encompass stimulus-reward association learning? Translated into functional anatomical terms the question is whether visual objects might gain motivational and emotional significance through an interaction between the IT cortex and the limbic system. Some preliminary findings supported this possibility. Earlier we had shown that equally severe deficits in object discrimination reversal learning are produced by bilateral removal of IT cortex and by bilateral removal of the temporal pole and amygdala (TPA), a limbic region to which the IT cortex projects. To test whether the similarity in outcome reflects a truly functional relation between the two areas, we prepared monkeys with an IT removal in one hemisphere, a TPA removal in the other, and a transection of the anterior commissure. The effect on object reversal learning was the same as that of the bilaterally symmetrical lesions suggesting that section of the anterior commissure had disconnected the intact TPA region on one side from the intact IT cortex on the other. Section of the corpus callosum instead of the anterior commissure was ineffective. The results thus supported the proposal that the process of associating visual stimuli with rewards depends in part on a visual-limbic pathway with a link in the IT cortex.

We are now extending those studies to exploit this new evidence of a visual-limbic interaction. First, we undertook a neuroanatomical investigation using the Fink-Heimer technique to define exactly the amygdaloid projections not only from visual cortex, but from all the telencephalic sensory systems. The results support the following conclusions: 1. All sensory systems contain areas that project to the amygdaloid complex (the somatosensory system, tentatively so), but not to more central limbic structures in the basal forebrain and hypothalamus. Consequently, whatever influence the sensory systems have on emotional and motivational processes mediated by these more central limbic structures is likely to depend largely on relays through the amygdala. 2. Except for the olfactory system, the amygdalopetal projections arise only from the later stages of cortical processing within each sensory system, i.e., from the modality-specific association areas one or more steps removed from the primary sensory areas. Thus, the modality-specific cortical sources of the amygdalopetal projections, like their amygdaloid targets, are important links in the sensory-limbic pathways. These sources are: for vision, areas TE and ventral TG; for audition, anterior TA and dorsal TG; for taste, area IA; and for somesthesis, possibly areas IA or IB. The amygdalopetal sources thus occupy a limited territory that begins dorsally in the anterior insula and extends ventrally across the anterior temporal neocortex as far as the rhinal fissure. 3. Within the visual system, progressively heavier and more widespread efferents arise from successively later stages of the amygdalopetal sources. The posterior half of TE sends a moderate project to the dorsal part of the lateral nucleus, the anterior half of the TE sends a heavy projection to the dorsal parts of both the lateral and basal nuclei, and the ventral part of TG sends a heavy projection to the dorsal and medial parts of the lateral and basal nuclei and to the dorsal part of the basal accessory nucleus. This pattern of progressive intensification and spread of the amygdalopetal projections applies also to the auditory system and probably to the other cortical sensory systems as well. The pattern suggests that a progressively greater influence on amygdaloid activity is exerted by successively more highly processed sensory information. 4. The efferents to the amygdaloid complex from the different sensory systems terminate in a dovetailed pattern. The major amygdaloid targets are: for vision, the anterodorsal parts of the lateral, basal, and basal accessory nuclei; for audition, the ventrocaudal parts of the lateral and basal accessory nuclei; for taste, the medial parts of the lateral and basal nuclei; and for olfaction, the cortical and medial nuclei. This pattern implies that each part of the amygdala is under the dominant influence of a particular sensory system.

The locus within the amygdala for the projection from the visual system was confirmed in the 2-deoxyglucose experiment described earlier. It would be of interest at some point to determine whether damage to just this part of the amygdala would produce the effects on visually mediated learning that have now been found after total amygdalectomy. This relates to the second way in which the visual-limbic interaction studies have been extended. Specifically, we have refined our behavioral assessment by replacing the discrimination reversal problem with a one-trial associative memory test analogous to the one-trial visual memory test described earlier. Whereas only IT lesions had impaired performance on the visual memory test, results with the associative memory test have indicated equivalent impairments after both IT lesions and lesions limited to the amygdala. Presumably, the impairment of the animals with IT lesions on the associative task was due to their basic recognition disorder found previously. The impairment in the animals with amygdala lesions, however, not being attributable to a recognition disorder, appears to reflect instead a disorder in object-reward association. The results thus point to object recognition and object-reward association as consecutive processes that depend in part on the sequential participation of IT cortex and the amygdala. An attempt will next be made to disconnect the amygdala from its IT input and then test the effects of this disconnection on associative memory.

Concurrently we will be testing directly whether the amygdala is involved in the establishment of stimulus-reward associations. The study involves measuring activity of single units in the amygdala while an animal performs a visual discrimination reversal. Positive evidence for our hypothesis would be provided by a binding that the discharge frequency of a given unit depends on the reward value of the stimulus (A+ vs A-) and not on its sensory quality (A vs B). The converse results, i.e., sensitivity to the sensory and not to the affective quality of the stimulus, would be expected of units in the IT cortex, for according to our model this tissue is an intrinsic part of the visual system and concerned with purely visual processing. Preliminary results have been gathered thus far on units in the IT cortex, and, as predicted, many of the neurons here discharge selectively to visual patterns - one cell preferring one subset of patterns, another cell preferring another subset, and so on - whereas few if any of these neurons appear to code the reward value of the patterns. Whether such a code is carried by a higher proportion of neurons in the amygdala still remains to be determined.

In the process of investigating the role of various temporal-lobe structures in the visual memory of the monkey we have chanced upon a result that is particularly exciting because it may help to solve a long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in animals by removal of the hippocampus have largely failed. What we have found in our studies, both for visual recognition memory and for object-reward association memory, is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss ensues, a loss that is even greater than that produced by lesions of IT cortex. The discovery not only opens up a new possibility for resolving the discrepancy between clinical and animal findings, it also provides a new insight into the neural mechanisms of memory. Pursuit of this line of study is now a major part of our program. For example, two new experiments have shown that the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems. In both experiments, rhesus monkeys were trained preoperatively on a one-trial visual recognition task requiring memory of single objects for 10 seconds each, retrained on this task postoperatively, and then given a performance test in which their one-trial recognition ability was taxed with longer delays (up to 2 minutes) and longer list lengths (up to 10 objects). Their average scores on this performance test were compared with those of previously studied groups that had been given either separate amygdaloid and hippocampal removals (average score, 90%) or combined removals (average score, 60%).

In the first experiment, bilateral amygdalectomy was combined with unilateral hippocampectomy, and vice versa. Both groups obtained average scores near 75%, midway between those of the comparison groups. The results support the view that the amygdala and hippocampus are equally important for recognition memory and that there is a quantitative relationship between the amount of damage to the two structures and impairment of this function. The same conclusion could apply to man.

In the second experiment, bilateral amygdalectomy was combined with bilateral transection of the fornix, a major hippocampal-diencephalic pathway; and bilateral hippocampectomy was combined with bilateral transection of the stria terminalis, a major amygdaloid-diencephalic pathway. Again both groups obtained average scores near 75%. These results point not only to joint participation by the amygdala and hippocampus in short-term memory, but to a wider, limbic-diencephalic circuit underlying this process.

Coincidentally, it was found in the cortico-limbic anatomical study described above that the same cortical areas that give rise to separate sensory channels to the amygdala send efferents that converge upon the perirhinal and prorhinal cortices, areas known to be a major source of input to the hippocampus. Consequently, both the amygdala and hippocampus can be activated by the same highly processed sensory information, a conclusion that may help to account for our finding that these two structures can substitute for each other in a mechanism for recognition memory.

Cerebral Mechanisms in Spatial Perception and Memory

The visual pathway described in the previous section is important for object vision, that is, for the analysis and coding of the physical dimensions of visual stimuli and for their identification through associations with other events, particularly motivational and emotional ones. There is a second major visual pathway out of the striate and prestriate cortex, however, and this one appears to play an important part in spatial vision, that is, in the analysis of the spatial location of objects and in the use of this information for the guidance of movement. The first pathway is directed ventrally to the temporal and frontal limbic systems. By contrast, the second pathway is directed dorsally and appears to provide access to the motor system. The evidence in support of this dichotomy of cortical visual pathways and the reasons for categorizing the second one as primarily spatial rather than purely visual in function come from recent experiments in our laboratory on the parietal and frontal lobes.

We had found earlier that bilateral posterior parietal lesions produced severe impairments on a spatial test in which the animal was required to choose a response location on the basis of its proximity to a visual "landmark". The effective lesions were large, invading not only inferior parietal but also dorsolateral and medial prestriate tissue. To test whether the responsible damage might have been to the prestriate rather than to the parietal cortex, new experiments were performed with more selective lesions. The results failed to reveal any localization within the larger region, and indicated instead that each part contributes to the function equally. The findings thus give added support to the view that the inferior parietal lobule, rather than being a modality-specific tactual association area as was once thought, is probably a multimodal area with a significant part played by vision. To test for the dependence of this area on visual input, a crossed-lesion study was undertaken. A posterior parietal lesion in one hemisphere was combined serially with a total striate removal in the other, and these were followed by callosal transection. Animals trained on the landmark test were severely impaired by the crossed lesions alone (performed in either order), and once recovered they were only mildly re-impaired by the callosal lesion. The results suggest that the inferior parietal cortex, like the inferior temporal, depends heavily on striate input for its visual function; but unlike the inferior temporal, the posterior parietal does not seem to receive a heavy ipsilateral visual input by way of the corpus callosum. Thus, each posterior parietal area may be organized largely as a substrate for contralateral spatial function, and this could account in part for the symptom of contralateral spatial neglect that has so often been reported after unilateral parietal injury.

In another experiment, monkeys received bilateral lesions of the striate areas representing either central vision (lateral striate) or peripheral vision (medial striate). They were tested both on a pattern discrimination task, to assess residual inferior temporal function, and on the landmark task, to assess residual posterior parietal function. On the former task, only the lateral striate lesion produced a deficit, whereas on the latter task, the two lesions produced equally severe deficits. Apparently, inputs from central vision are especially important for the pattern recognition functions of inferior temporal cortex, but inputs from central and peripheral vision are equally important for the visuospatial functions of posterior parietal cortex. Thus, although interactions with striate cortex are critical for the parietal just as for the temporal area, the striate inputs to these two regions appear to be organized differently: relative to inferior temporal cortex, posterior parietal cortex receives a greater contribution from inputs representing both the contralateral and the peripheral visual fields. These differences, which are seen also in the receptive field properties of posterior parietal vs. inferior temporal neurons, presumably reflect differences in the cortical processing required for spatial vs. object vision. Incidentally, a direct demonstration that the posterior parietal cortex is visually related was provided by the 2-deoxyglucose experiment described in the previous section.

The spatial functions of the parietal area have been categorized on the basis of our studies as "allocentric", where spatial location is defined in terms of referents external to the animal. Another form of spatial perception is "autocentric", where location is defined with reference to the animal's own body. Evidence is accumulating that this form of spatial orientation is dependent on dorsal prefrontal cortex, and that impairment in autocentric localization after dorsal prefrontal damage is the basis for the frontal animal's impairment on spatial delayed response tasks. (The dissociation between an allocentric and an autocentric spatial mechanism derives in part from our finding that parietal and prefrontal lesions each impair one mechanism but not the other.)

Crossed-lesion experiments like those described above have shown that the dorsal prefrontal cortex also depends on striate input for its visual spatial function. Evidence that the function is selectively spatial comes from a recent experiment in which animals with selective prefrontal removals were compared on a series of nonspatial visual memory tests. These included delayed object alternation, delayed color matching, and delayed object matching. In every case, animals with lesions confined to the cortex in the principal sulcus quickly reattained criterion performance, in sharp contrast to their notoriously poor performance on spatial visual memory tests. In another experiment we obtained evidence that autocentric spatial localization is probably not a purely kinesthetic function. On a test that required precise judgments of the extent of head or hand movements, prefrontal lesions alone produced little or no impairments. Since it is thus possible to spare kinesthetic discrimination ability while at the same time severely impair spatial perception, it is clear that the two abilities must be at least partly independent. At the same time, other evidence was obtained that with sufficiently large frontal lesions, which included both dorsal prefrontal and dorsal premotor cortex, a severe kinesthetic impairment could be produced. The findings thus point to a substrate for kinesthesis that is close to, yet separate from, the substrate for spatial perception. Indeed, it is reasonable to suppose that the system for autocentric localization depends on input not only from vision, but from kinesthesis and many other modalities, and, further, that the dorsal prefrontal area, like the posterior parietal, is a center for convergence of multimodal information organized in terms of some spatial coordinate system. The hypothesis suggests a new direction for electrophysiological studies of these dorsal cortical association areas.

It was noted above that the outputs of these dorsal association areas may be directed toward the cortical motor system and thus permit spatial guidance of movements. Disconnection experiments involving the spatial and motor systems, like those described earlier involving the visual and limbic systems, would provide a direct test of this proposal. Indirect support for this notion came from an unexpected source. In the previous section the evidence was reviewed showing that visual discrimination habits depend on both the cortical visual system and ventral limbic structures. But are these two systems acting together a sufficient substrate for such habits, or does the rest of the cortex, including that in the dorsal "spatial" system, also play a significant role? To examine this question we restricted vision to a single hemisphere (by visually deafferenting and disconnecting the other hemisphere), and then removed *all* the cortex from the seeing hemisphere except for the visual and limbic systems. Unexpectedly, the operated animals appeared on awakening to be completely blind, and this total blindness lasted for one to several months, abating, if at all, only very slowly and only in part. Yet, the geniculostriate pathway in these animals seemed to be intact as judged both by visual evoked potential studies (performed in collaboration with Buchsbaum, NIMH) and from the subsequent histological examination of the brains.

To determine whether the blindness was the result of a disconnection of the visual system in one hemisphere from the motor system in the other, animals were prepared as before but with the forebrain commissures left intact. Yet these animals too were blind, indicating that the blindness is not a disconnection effect and suggesting further that during blindness the visual signal is not processed beyond striate cortex, for otherwise the signal should have been transmitted across the preserved commissural channels (which start at the striate-prestriate border) to the motor system. The analysis implies that the territory ablated normally provides some activating input, direct or indirect, that is necessary for cortical visual processing.

In a further study, the original lesion was subdivided in an effort to localize the area supplying the activating input. It was found that a sensorimotor lesion (Brodmann's areas 1-6) failed to produce blindness in any of three monkeys. The complementary lesion, however, consisting of dorsal prefrontal, inferior parietal, insula and superior temporal areas, did produce blindness in three monkeys for about a month. These localization results provide additional evidence that the blindness is not a visual-motor disconnection effect, and suggest instead that it is a profound form of visual neglect, since parts of the lesion that produced blindness are known to produce neglect. Accordingly, the basis of the neglect too could be impaired processing within the cortical visual system due to loss of activating input, though a less complete loss than that producing neglect.

Monkeys prepared with the standard lesion were behaviorally blind for periods ranging from 25 to more than 400 days. The extreme variability in the duration of the blindness has not only been puzzling but has impeded further analysis of the phenomenon. New studies indicate that this variability can be eliminated and a predictably long-term effect produced in two different preparations.

First, the original findings suggested a species difference that has now been confirmed with additional animals given the standard lesion. Thus, whereas a total of four rhesus monkeys (Macaca mulatta) have recovered from blindness after a median of only 28 days, none of four cynomolgus monkeys (Macaca fascicularis) has yet shown recovery despite a median postoperative survival period of 180 days.

Second, the rhesus monkey also will show prolonged blindness if the standard lesion is expanded to include most of the limbic cortex. Three animals prepared in this way have thus far remained blind for a median period of 100 days with no signs of recovery. The limbic lesion alone will not cause this effect as evidenced by two monkeys with such a limbic ablation that demonstrated accurate visual guidance immediately after surgery.

We had proposed on the basis of the previous evidence that the blindness results from a failure of visual processing within the intact visual system due to the loss of an essential activation normally provided, directly or indirectly, by the territory included in the ablation. The newest findings strengthen this hypothesis by rendering highly unlikely the alternative possibility that the blindness reflects some transient state such as surgical shock or diaschisis. We are now testing our hypothesis directly by examining stimulus-evoked neuronal activity within the intact visual system of chronically blind animals.

It appears from these results that the dorsal "spatial" system normally provides an activating input that is necessary for the sensory systems to function properly, and that this is in addition to its contribution to spatial mechanisms. For the animals that eventually do recover their use of vision then show permanent impairment in the ability to guide their limbs to visual targets; that is, they then show the expected spatial-motor disconnection. Further studies dealing with both the spatial and the activating functions of the posterior parietal and dorsal prefrontal areas are now underway.

Development and Plasticity of Primate Frontal Lobe

The major goal of the Section on Developmental Neurobiology is the elucidation of basic principles governing the development and plasticity of prefrontal granular cortex in primates. The research program is composed of three integral parts: (1) analysis of the development and organization of certain classes of prefrontal cortical connections; (2) analysis of the functional maturation of the prefrontal cortex and related structures; and (3) assessment of structural and functional plasticity which follows early injury to the prefrontal cortex. The goals of this multifaceted project are achieved by a broad multidisciplinary approach including behavioral, anatomical, electrophysiological and biochemical techniques. These techniques are used in conjunction with recently refined procedures that permit prenatal neurosurgery on the primate brain with return of the fetus to the uterus and survival to and after term. The studies are conducted on the rhesus monkey because the expanse of association cortex in this species as well as its neuronal organization, protracted development and capacity for complex behavior makes it an excellent animal model for the study of normal and pathological development of human neocortex.

Organization and Development of Prefrontal Connections

Selective Association of Prefrontal Cortex with the Limbic System

Although one of the distinctive features claimed for prefrontal granular cortex is its intimate association with both cortical and subcortical components of the limbic system, information on the organization and topography of these connections is still not sufficiently detailed. Our anatomical analyses of prefrontal cortex in the primate brain have begun to provide new and important information about this significant class of prefrontal-limbic connections, particularly about those neuronal circuits which include the amygdala.

On the basis of both autoradiographic and histochemical analysis, we found that the primate prefrontal cortex of rhesus monkeys may be divided into two sharply segregated systems: i) a ventromedial system which constitutes the entire prefrontal projection of the amygdala and ii) a dorsolateral system which is essentially devoid of amygdalo-fugal projections. The ventromedial subdivision seems to correspond to the prefrontal cortex of the rat in which projections from the amygdala, the mediodorsal nucleus of the thalamus and the ventral tegmentum overlap. However, there appears to be no obvious homologue in the rodent comparable to the extensive amygdala-free dorsolateral convexity so prominent in primates.

Bilaterality of the Cortico-thalamic Pathway

It is generally considered that the mammalian neocortex projects exclusively to the ipsilateral thalamus. This view is based largely on studies using reduced silver impregnation methods for staining normal or degenerating fibers. Recently developed techniques for tracing neuronal pathways using anterograde axonal transport of labeled macromolecules have proven to be useful for more detailed analysis of central nervous system connections. Using this method following injections of radioactive tracers in the frontal lobe of the rhesus monkey, we observed a distinct bilateral distribution of cortico-thalamic terminals.

Cortico-thalamic efferents have been recognized for their strategic importance in mediating cerebral cortical control over incoming sensory input that converges upon the dorsal thalamus. The present findings indicate that in the monkey the cortex of one hemisphere may exert such influence over the contralateral as well as the ipsilateral thalamus. It may be wondered whether similar bilateral cortico-thalamic representation exists in man, in whom some regions of each hemisphere have become dominant for specific functions. Bilateral cortico-thalamic projections could provide a mechanism for such regions situated in one hemisphere to exert control over thalamic input on both sides of the brain.

Prenatal Development of Prefrontal Connections

Our studies on the formation of prefrontal cortical connections have focused this year on the callosal pathways which interconnect the two hemispheres and on the cortico-striatal projections. We have previously shown that the terminals of both classes of connections display a highly organized modular distribution in the adult brain. In addition, we have discovered that the cortico-striatal pathway exhibits a remarkable degree of reorganization in response to brain injury. The purpose of the studies that we conducted in the past year was to determine i) the timing and sequence of outgrowth of these classes of connections; ii) the mode of their formation into modules; and iii) whether the cortico-striatal projection is distributed bilaterally at early stages of gestation and later becomes mainly ipsilateral. The latter possibility is one of several mechanisms that I proposed to account for the rearrangement of fronto-striatal connections that we found after unilateral resection of the dorsolateral prefrontal cortex at midgestation.

Our preliminary findings indicate:

(1) The principal long-tract pathways of the prefrontal cortex are layed down well before birth in the rhesus monkey. This was suspected, of course, but not really known. The callosal pathway enters the homotopic cortex in the contralateral hemisphere between E95 and E155; the cortico-striatal pathway forms earlier between E69 and E95. However, further experiments in more closely spaced fetuses are necessary to determine the precise timing and sequence of innervation.

(2) Columnar organization of the callosal pathways is achieved at least two weeks before birth and perhaps somewhat earlier. This precocious appearance of modular organization in the connections of the frontal lobe contrasts with the postnatal formation of ocular dominance columns in the primary visual cortex of the same species.

The cortico-striatal pathway develops in at least two broad phases: in the first phase, axons from the prefrontal cortex are distributed uniformly throughout the caudate nucleus; in the second phase these axons appear to become redistributed into an intricate pattern in which clusters of prefrontal fibers surround round or elliptically shaped cores that are devoid of axons from prefrontal cortex. This mode of formation of prefronto-striatal modules is similar to the pattern of genesis of retino-geniculate, retino-tectal and geniculo-cortical fibers in the primate visual system. The concordance of findings from studies of descending projections from frontal association cortex with those from studies of primary sensory systems suggests that the biphasic diffuse-to-specific scheme may represent a general principle governing the formation of all central nervous pathways.

Functional Development of Prefrontal Cortex and Related Structures

Electrophysiological Activity of Prefrontal Neurons

Previous research in this laboratory has provided evidence that specific regions of the primate prefrontal cortex develop their functions at different rates and according to different time-tables of maturation. In the last several years we have studied the maturation of function in the dorsolateral prefrontal cortex by detailed electrophysiological analysis of prefrontal unit activity at different stages of development. This year we have focused on the classification of neuronal subtypes within this same region with the ultimate goal of relating functional classes to the underlying laminar and modular organization of the prefrontal cortex.

The electrophysiological studies are performed on rhesus monkeys that have been trained to perform cognitive delayed response tasks requiring the integrity of the prefrontal cortex as well as on a number of control tasks that do not. After the animals are well trained, chronic indwelling cylinders are implanted in their skulls over the dorsolateral prefrontal cortex. Following recovery from surgery, neuronal activity is recorded while the monkeys are performing one or another task.

Our principal findings this year pertain to the types of activity that are found in frontal granular cortex and their relationship to behavior. The major findings are: (1) Firing changes occur most frequently during the early phases of the delay in a delayed response trial; (2) prefrontal neurons exhibit their greatest sensitivity to spatial location during the same early phases of the delay, i.e., a neuron may fire preferentially to a stimulus on the left (as compared to a stimulus on the right) or vice versa most often during the early delay; (3) Differences between experimental and control tasks are sharpest when neuronal activity under the two conditions is contrasted during the early phase of the delay. These analyses confirm and extend previous findings that the prefrontal neurons are specialized for coding spatial-mnemonic information of a high order of complexity and that a critical process (encoding or registration) underlying this form of information processing occurs during the early phases of the temporal interval over which information must be retained.

Biochemical Analysis of Development of Monoamines in Prefrontal Cortex

Studies on the development of monoamine systems in major cortical and subcortical structures of the primate central nervous system continues to be a major focus of our activities this year. Over the course of this year we have added a number of additional animals and age-points to our previous analyses. However, our recent findings which employ the methods of cation exchange chromatography and fluorimetric assay confirm our previous results that were reported last year. Briefly, there are considerable differences in the time-tables of development of dopamine, norepinephrine and serotonin in different regions of the nervous system. We have identified three major periods of maturation which span a) a neonatal stage from birth through 8 months of age; b) a juvenile stage from 8 months to 2-3 years of age; and c) maturity which beings at 2-3 years and extends to 15 years and over, which in rhesus monkeys can be considered the equivalent of old age in man. Since the changes in brain chemistry with age are quite complex, it will be convenient to describe the major findings for each putative transmitter separately.

Dopamine is found in high concentrations in the prefrontal cortex of newborn monkeys. However, the level and rates of synthesis of this amine decline over the neonatal period from birth through 8 months of age. In contrast, dopamine content and activity in motor cortex increase over the same period, reaching a peak by 8 months of age. The decline in prefrontal dopamine and the increase in precentral dopamine may possibly reflect a redistribution of dopaminergic activity within the frontal lobe over the neonatal period of postnatal development as well as maturation of control mechanisms for dopamine synthesis and regulation. Peak-levels of dopamine that are attained in motor cortex in the first eight months may be related to the increase in motor dexterity and fine coordination that begins to be evident at this age. Dopamine content and activity in prefrontal cortex increases from 8 months to 2-3 years of age followed by a marked decrease in aged monkeys. The increase in prefrontal dopamine at the stage of sexual maturation correlates with our independent evidence from studies of surgical and reversible lesions and from electrophysiological data which indicate that the dorsolateral prefrontal cortex reaches maturity for certain cognitive functions at this age. The decline in dopamine in old-aged animals (which is restricted only to prefrontal cortical dopamine) may be related to the decline in cognitive efficiency that sometimes occurs in old age. If so, reversal of such disorders may be possible with dopamine agonists.

In contrast to dopamine, norepinephrine exhibits a steady and graded increase with age in both levels and biosynthetic activity in all areas of the brain where it is present in measurable concentrations. It is noteworthy that norepinephrine which is present in peak concentrations in somatosensory cortex at all ages falls off sharply in the 15 year old monkeys. Norepinephrine content of other cortical and subcortical regions is only minimally affected by old age.

Serotonin is rather uniformly distributed throughout the neocortex at all ages but its metabolite, 5-HIAA, is present in higher concentrations in posterior regions of the neocortex. Developmentally, serotonin synthesis rates are low in parietal and visual cortex over the neonatal period from birth through 8 months. However, between 8 months and 2-3 years, there is a 75% increase in synthetic activity in these regions, thus indicating an increasing serotonergic influence of the functions of parietal and visual cortex with maturity.

While the functional role of monoamines in neocortical activity and in development of such activity needs to be elucidated, the dynamic and regionally specific changes in monoamine concentration and activity suggests a differential role for the different transmitters in each area of the cortex. Further, the functions mediated by each cortical area may well be modulated by a different neurotransmitter at each developmental time period. Further studies are required to decipher the meaning of these shifts in neurotransmitter balance over various stages of the life span and to explore their significance in pathological as well as normal behavioral development.

Seasonal Variation in Monoamines in Primate Hypothalamus

In the course of our development studies of monoamine systems we made the surprising discovery that serotonin levels and rates of utilization exhibit marked seasonal variation in the hypothalamus of rhesus monkeys. This finding confirms and extends recent results obtained in postmortem studies of human subjects in Sweden .

Using cation exchange chromatography and spectrophotofluorimetry, we found that serotonin concentration in hypothalamus is very high in summer and lower in winter. Further, levels of 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptophan accumulation after synthesis inhibition parallel the seasonal variations in 5-HT, thus indicating that utilization parallels synthesis. In addition there may be an interaction between the catecholamines and indoleamine systems: when dopamine catecholamine synthesis is high, serotonin synthesis is low and vice versa. Further studies are needed to fully assess the functional and biomedical significance of such biochemical rhythms in the primate diencephalon.

Structural and Functional Plasticity of Primate Telencephalon

Rearrangement of Connections Following Resection of Prefrontal Cortex

A major goal of our efforts this year was to extend information on the reorganization of the cortico-striatal pathway that occurs following resection of the dorsolateral prefrontal cortex in one hemisphere 6 weeks prior to birth. Specifically, we are examining; (1) whether there is a critical period for the induction of anomalous neuronal pathways in the primate telencephalon; (2) whether abnormal reorganization of connections is a property of the cortico-striatal pathways exclusively; and (3) the relationship between anatomical reorganization and recovery of function after cortical lesions.

To shed light on these issues, we have performed unilateral cortical resections in fetal monkeys at various stages of gestation and at different stages of postnatal life. In the case of prenatal surgery, the fetus is exteriorized following a laparotomy and hysterotomy performed on a timed-pregnant rhesus monkey. Cortical tissue in the inferior bank of the primordia arcuate sulcus and in both banks of the developing principal sulcus and all cortex lying on the dorsal convexity superior to the principal sulcus is ablated. After the surgery is complete, the fetus is returned to the uterus and allowed to survive until term which occurs on average at 165 days of gestation. At term, the fetus is delivered by Caesarean section if delivery is not spontaneous.

Our experimental plan is to assess these monkeys subjected to unilateral cortical resection at various stages of pre- and postnatal development on a battery of behavioral tests. Such testing requires more than one year to complete per animal. Following the termination of behavioral assessment, the animals will receive injections of tritiated amino acids into the principal sulcus in the hemisphere opposite to that previously resected. Since the subjects for anatomical analysis are still being tested behaviorally we have not completed the anatomical studies as yet. However, preliminary findings indicate (1) that we may anticipate alterations in a number of classe of prefrontal connections; (2) these alterations can occur following unilatera resection at various ages ranging from two months before birth until two months after birth; (3) the degree of axonal reorganization may be correlated with recovery of behavioral functions.

Neurological, Neuropathological and Behavioral Effects of Thiamine Deficiency in the Rhesus Monkey

Another example of functional plasticity of the primate central nervous system has come from our recent studies on the behavioral and anatomical consequences of thiamine deficiency in the adult rhesus monkey. Thiamine deficiency is thought to be an etiologic factor in Wernicke-Korsakoff Disease.

Seventeen adult male rhesus monkeys were divided into five groups: Thiamine Deficient One Bout Group (TD1), Thiamine Deficient Two Bout Group (TD2), Thiamine Deficient Four Bout Group (TD4), Pair-fed Control Group (PFC), and Normal Control Group (NC). The animals in groups TD1, TD2, and TD4 were subjected to one, two, or four periods of thiamine deficiency, respectively, alternating with thiamine administration and recovery periods. The three PFC animals were matched to an animal in each of the three experimental groups (TD1, TD2, TD4) and given the same quantity of experimental diet as that animal, but were supplemented with thiamine. NC animals received Purine Monkey Chow ad. lib. Following the deficiency periods, experimental and control animals were tested on four behavioral tasks: object reversal, place reversal, delayed response, and delayed nonmatching-to-sample. Results showe that, regardless of the number of periods of deprivation, thiamine deficiency produced neurological symptoms such as anorexia, lethargy, ataxia, and ophthalmoplegia similar to those found in Wernicke-Korsakoff Syndrome. The histopathological changes consisted of well circumscribed bilaterally symmetrical lesions located in the basal ganglia, the parafascicular nucleus of the thalamus, the periaqueductal gray matter of the midbrain, the pons, structures in the floor of the fourth ventricle, and the roof nuclei of the cerebellum. The severity of the damage and the topographical distribution of the lesions varied with the number of periods of deficiency. Lesions were not found in the mammillary bodies or the medial dorsal nucleus of the thalamus, structures that are thought to be important for the memory impairment in Korsakoff patients. Nevertheless, the thiamine deficient animals showed a selective impairment on the delayed nonmatching-to-sample task. This impairment was not related to the number of periods of deficiency, but rather to location of pathological lesions. Nevertheless, one to four periods of thiamine deficiency is not sufficient to produce all of the clinical, histopathological, and behavioral symptoms characteristic of Wernicke-Korsakoff Syndrome. Altogether the evidence suggests that more prolonged deficiency is necessary to elicit the full range of clinical symptoms of this disease.

Annual Report of the Laboratory of Psychology and Psychopathology National Institute of Mental Health

October 1, 1978 to September 30, 1979

Herbert Weingartner, Ph.D., Acting Chief

The past year was one that can be viewed as the initial stage in rebuilding the Laboratory of Psychology and Psychopathology. At the same time, current research activities were expanded resulting in some important new research contributions which will increase our understanding of normal and disturbed behavior.

During the past year, the Laboratory has also initiated new areas of study, as well as productively expanding research areas that have been part of past LPP activities. These research findings, summarized here, have important implications for defining the psychobiological determinants of psychopathology. These research efforts and findings are also important for our understanding of normally functioning behavioral systems. LPP research activities have been concentrated in the following areas:

1. Psychobiology of cognitive processes.
2. Psychophysiological (autonomic nervous system) responsivity as it relates to personality and psychopathology.
3. Genetic, environmental and neurophysiological factors in psychiatric illness.

Psychobiology of Cognitive Processes

Research projects have been designed to study the psychological and biological mechanisms involved in attention, acquisition (learning) memory and retrieval of experience. Many of these studies have involved drug-induced changes in various psychobiological components of cognition in psychiatric populations, such as in mood-disordered patients, neuropsychiatric disorders and hyperactive children. Some of the findings summarized here are useful in better understanding the structure of cognitive disorders that are a major expression of many psychiatric disorders. Findings have also been important in the development of new treatment strategies. At the same time, findings provide new information about the mechanisms that determine aspects of normal cognitive functioning.

In a number of experiments we have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and its retrieval. Arecoline, physostigmine, and lecithin treatments produce enhancements in the storage and retrieval of information. Scopolamine treatments which disrupt cholinergic activity, produce an impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine-induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated patients with Huntington's Disease. In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in an undrugged state, and afterwards are treated with arecoline, there is a comparable facilitation of information later recalled, when compared to learning and recall that occurs under arecoline treatment conditions. While cholinergic treatments alter how much information is stored and later retrieved from memory, these effects are also state dependent. That is, there appear to be qualitative cholinergic drug state specific differences in how experiences are encoded and how retrieval of past experience is accomplished.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input process under drug-state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (the size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition in depressed patients, hyperactive children, normal children, and normal adults. Amphetamine also induces a change in state which serves as a state specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state dependent learning and state dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine and the cognitive disrupting effects produced by scopolamine vs. lithium has served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

Some behavioral strategies have also been used to investigate the specificity of cognitive processes as these might be involved either separately or together in the acquisition, elaboration and amplification, trace maintenance in memory, and retrieval. The emotional properties of stimuli seem to amplify or strengthen what is learned or stored in memory in a manner similar to that produced by drug treatments such as amphetamine. Reinforcement following information processing also acts to strengthen events already in memory storage. Other behavioral determinants, such as word imagery, presentation rate, familiarity, stimulus structure, appear to alter how much is learned, rather than the strength of learning, in a manner similar to the effects of increased cholinergic activity.

Clinical studies have been designed to investigate the specificity of changes in components of cognitive processes in adults with disturbances in mood, schizophrenic patients, hyperactive and learning-disabled children and patients with different forms of dementia. The studies that have contrasted the different forms of disrupted cognition in Huntington's Disease (HD), Korsakoff's Psychosis, Alzheimer's Disorder and other neurological disorders. Findings include 1) HD patients fail to consistently remember information which is, nevertheless, in memory store; 2) HD patients demonstrate retrieval failure which is due to inadequate encoding of information during acquisition. They fail to use slow presentation rate, semantic encoding, imagery and other attributes of stimuli to more effectively and elaborately encode information. Some drug and behavioral treatments may prove helpful to normalize this encoding disruption; 3) Alzheimer's patients also fail to "appreciate", learn and retrieve experiences. These patients have access to appropriate semantic structures in memory but fail to use these in organizing experiences so that they might prove memorable; 4) Alzheimer's patients appear to show a small but measurable cognitive response (improvement) following cholinergic and amphetamine treatment. Recall of organized information is most facilitated while rote-like learning seems unaltered. It is as yet uncertain whether the cholinergic response is qualitatively different from that seen following amphetamine treatment. These patients have also been treated chronically with a cholinergic drug, have appeared to show a temporary improvement in cognitive functions but this enhancement is not a sustained drug response. Strategies for defining cognitive changes in Alzheimer's Disorder, and potential treatment strategies are relevant issued in the study of normal aging process; 5) Recent studies testing the cognitive changes that follow treatment with vasopressin-like compounds (DDAVP) may provide us with some new strategies in the treatment of dementias. We have shown that DDAVP produces a marked and sustained cognitive enhancement in depressed patients, as well as in normal controls. The form of that cognitive enhancement appears to act through altering organizational mechanisms which may be particularly relevant for the kinds of disruptions in cognition seen in Alzheimer's patients. Studies of the structure of cognitive changes in response to neuropeptide treatment should prove helpful in defining biological mechanisms that are important for those cognitive processes involved in the organization and elaboration of information in memory; 6) Recently completed cognitive studies of depression have shown that these patients demonstrate learning memory failures when they are required to organize information, but show no such deficit when organization is present. That is, when information is presented in an organized fashion, then depression-related cognitive deficits are not apparent. In some respects, their cognitive disruption is similar to that seen in Alzheimer's Disorder. These patients also demonstrate qualitative changes in how they encode events and the strategies by which they recollect previously learned experiences (state-dependent learning). Some of these effects may be related to alterations in lateralization of cognitive processes; 7) Studies of cognitive processes in children have demonstrated some of the specificity of cognitive disturbances that are apparent in hyperactive and learning-disabled children. Hyperactive children appear to learn (store) about the same amount of information as normal controls (under experimental conditions). However, unlike controls, retrieval of that information is highly dependent on the presence of strong environmental cues or prompts at the time remembering takes place. Both normal and hyperactive children respond with enhanced cognition following amphetamine treatment. However, those aspects of cognition that are enhanced in these different children are qualitatively different. The facilitation of cognitive functions in the hyperactive child is one which would not readily translate into more effective school performance, since amphetamine increases learning and recall of superficial characteristics of information processing. This is in contrast to an amphetamine-induced increase in learning and recall of meaningfully processed information in the normal child. It therefore appears useful to integrate behavioral retraining strategies along with stimulant treatment to effectively help the hyperactive child; 8) Recent studies examining learning and recall in stimulant-treated hyperactive children have also shown that learning and retrieval of learning are stimulant drug-state specifi (state-dependent learning). Although learning may be enhanced following amphetamine treatment, its effective retrieval is largely dependent on reinstitution of the drug-state present at the time of learning. This has clear implications for the long-term effects of stimulant treatment in hyperactive children, particularly in how this affects their school performance.

Psychophysiological Responsivity, Personality and Psychopathology

The general aim of this research is to investigate various measures of autonomic nervous system activity (ANS) and relate such responsivity to attentional processes in normal man and in psychopathology (e.g., focusing behavior in schizophrenia, hyperactive children, as well as in relationship to various measures of personality in psychopathology). In a series of studies, it was observed that autonomic nervous system activity is related to attentional disturbances. This pattern of dysfunction has been characteristic of chronic schizophrenics, and distinguished unmedicated acute schizophrenics, who subsequently had a remission of symptoms, from those who remained psychotic after a four-month period of hospitalization. These laboratory measures of behavior appear to be markers of vulnerability to a more chronic course of the illness, while a more normal type of ANS activity and attentional functioning may assist in predicting aspects of the recuperative process.

The effects of d-amphetamine on ANS functioning and attention were observed in both normal and hyperactive children, as well as in normal adults. Improvements in attention following drug treatment were found in both groups of children and in adults. In normal adults this effect depended on drug dosage and attentional load of the task. Autonomic nervous system responses to d-amphetamine were fairly similar in all groups. Experimental results showed that the beneficial effects of stimulant drugs in hyperactive children are not paradoxical in the sense of being unique to hyperactive children. It was also found that, in children, d-amphetamine increased autonomic nervous system arousal but this was not reflected in changes in attention. This confirms the widely held notion that the behavioral effects of stimulant drugs are due to their effects on arousal system.

Various measures of personality seem to be correlated with psychophysiological responsivity and performance on various perceptual tasks. Subjects that were classified on the basis of various personality measures which defined aspects of psychoticism, extraversion, introversion, sensation seeking, field dependence, ego strength, and impulsivity show systematic differences in their sensitivity to auditory and visual stimuli as measured by threshold values, two-flash thresholds, fusion and absolute auditory thresholds and by measures of psychophysiological responsivity to these stimuli. Racial differences in ANS responsivity between black and white subjects were also observed in sensory processing and arousal. The major thrust of this research has been to demonstrate relationships between measures of autonomic nervous system activity, personality and perceptual processing.

Another series of studies has involved an attempt to look at the relationship between personality factors, biochemical measures, and psychophysiological variables in normal college students. This research has involved an 18-month follow-up of a large group of normal student volunteers. Some of these students reported an occurrence of major depression or hypomania during the follow-up period, were found to have a combination of either low-MAO and augmented evoked response (EEG) or high-MAO and reduced evoked responses. There appear to be biological parallels between psychiatric patients and non-hospitalized individuals vulnerable to psychopathology. In another study, 400 college students were screened on measures of attention. Subgroups of these students were selected on the basis of either good or poor attention measures. These subgroups were then contrasted in terms of I.Q., the presence or absence of psychopathology, aspects of learning and memory and neurological signs, as well as MAO and psychophysiological responsivity. In a preliminary analysis of these findings, it appears that a history of hyperactivity is related to poor attention in this population of young adult college students. No differences between the groups were apparent on psychopathological or neurological signs.

Genetic Studies

LPP research has continued to critically examine, contrast and discriminate between hereditary and environmental determinants of schizophrenia and other psychiatric disorders. Much of this research has suggested that hereditary factors do contribute to the development of schizophrenia and to other disorders that we include in a group called the schizophrenia spectrum. Other related research findings suggest that the offspring of schizophrenics have more neuropathological signs in childhood than do controls. Further research in this area has been designed to 1) discriminate gene carriers and non-gene carriers with respect to personality and psychological test variables; 2) compare the fate of gene carriers who are reared in parental homes in contrast to those reared in adopted homes; 3) compare subjects who have a psychotic biological parent, but who are reared by non-psychotic parents whose biological parents are not psychotic but who are reared by a psychotic adoptive parent. Research efforts have attempted to find the incidence of the schizophrenia spectrum disorder in a random sample of subjects (in a Danish population).

Additional studies of genetic factors in psychopathology have been studied by examining the biochemical and related behavioral variations in lower animals. Presently, this objective is being pursued through a study of normal variation in biogenic amine metabolism in mice and rats. Previous studies have indicated constant levels of MAO activity across age (100, 200, 300 and 400 day-old mice were studied) when serotonin and phenylethylamine are used as substrates but an unexpected decrease in tyramine deamination in 400 day-old subjects. The repetition of this age study with the addition of a group of 500 day-old subjects is in progress.

Variability in MAO activity was demonstrated among inbred mouse strains but the nature of the inheritance of these differences has not been studied. The rank ordering of the strain means suggests a high correspondence between liver and brain MAO activity as well as a high positive association between the activities of the A and B forms of MAO, as measured with the substrates phenylethylamine, serotonin and tyramine. Environmental variables and genotype environment interactions have also been found to be of importance in controlling activity levels of MAO. Increased levels of MAO-B but not MAO-A were found in livers of Brown Norway Rats but not in Wistar Kyoto Rats after two hours of stress. Conversely, the nature of the change in plasma dopamine-β-hydroxylase (DβH) activity in these same animals after two hours of stress was the opposite, i.e., there was no change in plasma DβH in the Brown Norway Rats but there was a substantial change in plasma DβH activity in the Wistar Kyoto Rats.

Neurochemical genetic variation is also being investigated in the form of plasma DβH variation in inbred mice and rats. Twenty strains of inbred mice have been screened for plasma DβH and large differences have been demonstrated among the mouse strains but intrastrain variability has been substantial. Twenty strains of inbred rats are now being examined. Rodents have low levels of plasma DβH activity. Assay revisions are being attempted to improve the reliability of the radiochemical two enzyme procedure for the measurement of low levels of enzyme activity.

Neurophysiological and neuroanatomical studies have explored possible CAT-scan abnormalities in schizophrenic and depressed patients. Findings from this study have also been used to develop strategies for discriminating between subgroups of schizophrenic patients and other psychiatric disorders. The aim of such subgroup discrimination is to relate specific forms of psychopathology to discrete biological/psychological determinants. It does appear that neuroanatomical abnormalities are present in chronic schizophrenic patients as revealed by CAT-scan analyses. These CAT-scan abnormalities include dilated ventricles, enlarged sulci, cerebral atrophy, reversals of typical lateral asymmetries. A number of studies have pursued how these CAT-scan abnormalities might relate to psychological test performance (WAIS I.Q., Halstead-Reitan Test). In addition, some attempts have been made to measure the frequency of these CAT-scan abnormalities among depressed, as well as schizophrenic patients. Direct data on genetic heterogeneity in schizophrenia is difficult to develop. Recently compiled data defining the differential response to neuroleptics of schizophrenics having affectively-ill relatives vs. schizophrenics having schizophrenic relatives, have been analyzed. Findings indicate that many "symptomatic schizophrenics" may share the same genes that lead to affective illness, and that the recovery of these schizophrenics may be inhibited by the neuroleptic medication which is commonly prescribed.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH
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Melvin L. Kohn, Ph.D., Chief

In social science research, initial findings are likely to come from a cross-sectional study of a single, usually narrowly delimited, population. Only later are these findings tested in studies of other delimited populations, in larger-scale studies, in longitudinal extensions of the research, and in cross-national replications. In recent years, an increasing proportion of the research done in this Laboratory has been longitudinal, reflecting our desire to verify the causal processes implied in the findings that have come out of our cross-sectional studies. This development will be apparent again in this report. This year we can also describe the first findings of a cross-national replication in Poland of one of our major studies and the prospect of another replication of this research in Japan.

These cross-national studies represent, in a new form, a resurrection of what I have long considered to be one of the most valuable facets of the Laboratory's research program, distinguished in earlier years by Leonard Pearlin's comparative study of class, occupation, and family in the U.S. and Italy, by William Caudill's and Carmi Schooler's comparative studies of parent-child relations and childhood personality development in Japan and the United States, and by Schooler's Japanese studies of the social determinants of values and role preferences in Japan. These studies, of great use in establishing the generalizability of our findings to other social and cultural contexts, seemed largely to have ended with the war in Viet Nam and the increasing difficulty of obtaining funds to do research abroad. In fact, Caudill's last period of fieldwork in Japan, and Schooler's Fellowship at the Japanese NIMH, were both financed by agencies of the Japanese, not the U.S., Government.

Now we seem to have evolved a new mechanism for cross-national comparative research, initiated by Wlodzimierz Wesolowski of the Polish Academy of Sciences. This mechanism has only limited applicability -- it can be used only when investigators abroad wish to replicate our research or we, theirs -- but, where applicable, it has great utility. Wesolowski proposed, to us and to the Polish Academy of Sciences, that a group of his colleagues carry out in Poland a study designed to be as comparable as possible to our research on the psychological effects of social class and occupational structure. The intent was to see if our results could be generalized to a socialist society, an issue of obvious importance both to Polish and to U.S. sociology. Wesolowski deliberately did not ask us to provide funding (which we could not in any case have provided), because he wanted the study to be a Polish, not an American endeavor. He did propose that the principal investigator, Kazimierz Slomczynski, come to the Laboratory for six weeks to plan with us the design of the study and the translation of the questions.

In further evolution of the arrangement, we invited Slomczynski to return to the Laboratory as a Visiting Scientist, after the data had been collected, coded, and edited, for collaborative analysis. What we could offer, in addition to the appeal of a year at NIH, was the opportunity to work closely with the original investigators, a considerable advantage in a replication study, because many ambiguities of intent and of research design can be clarified in the course of such collaboration. What Slomczynski could offer, of course, was an opportunity for us to partake in the analysis of this extraordinarily valuable body of data, in collaboration with a distinguished student of Polish society. That analysis is now in process. Slomczynski and his Polish colleagues will of course continue to analyze the data, much more extensively, after he returns to Poland.

Meantime, Kenichi Tominaga of the University of Tokyo, on learning of the Polish study and after exploring mutual interests with us, decided to make a three-way comparison of the Polish-American venture -- motivated in part by the rare opportunity to compare Japanese society not only to American society (as is typically done), but also to a socialist society. To both the Polish investigators and us, the opportunity to establish the generalizability of findings afforded by a comparative Japanese study is welcome, for two distinct reasons. Not only is there the cultural difference between Japanese society and both Polish and American societies, but Japan also has an unusual industrial structure, with a primary sector that provides an exceptional degree of job security and attachment of the individual to the firm or enterprise, and a secondary sector that provides little of either. The Japanese study follows an administrative pattern similar to that of the Polish: the inquiry is financed in Japan from Japanese sources; the study is theirs, not ours; but we have participated in planning the inquiry and verifying the accuracy of translation. In particular, Carmi Schooler spent five weeks in Japan doing intensive planning and translation. Our intent is to follow the Polish pattern, with data-collection, coding, and editing to be done in Japan, and then Atsushi Naoi coming to NIH as a Visiting Scientist for collaborative analysis of the data. As in the Polish study, the expectation is that the Japanese investigators will do much more extensive analysis of their data after returning home.

THE POLISH REPLICATION

The main purpose of this study is to examine the relationship of social class and occupational conditions to psychological functioning in a socialist society. Specifically, the study attempts to test, in a different economic, political, and cultural context, the central finding of Kohn and Schooler in the U.S. that occupational self-direction plays a crucial role in explaining the impact of social class on parental values, orientation toward self and society, and intellectual flexibility. The three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, submitted a research proposal to the Polish Academy of Sciences, the sponsor of the research, which was approved in

January 1978.

In comparison with the American questionnaire, the Polish version was extended by including several questions about membership in voluntary organizations and social participation. It was expected that certain differences in psychological functioning would be explained by involvement in the workers' self-management movement.

The Polish national sample consists of 1600 males, aged 21-65, living in urban areas, and presently employed full-time in civilian occupations. A subsample of 400 men was selected for additional psychological testing, using the Embedded Figures Test and the Draw-A-Person Test. Another subsample of 400 was selected for a reliability study.

The preparation of the replicative part of the questionnaire consisted of four steps: translation of questions from the American questionnaire and selection of the best Polish version of each question; a pilot study; modification of the questionnaire after the pilot study; and testing the final version.

The first version of translation of each question was prepared by Slomczynski during a six-week stay at the Laboratory of Socio-Environmental Studies in 1976. Discussions with members of the Laboratory helped Slomczynski clarify the meaning of entire questions, special phrases, and, especially, questions that include colloquial expressions, such as "going to pieces", "end up causing trouble", "to feel upset". One difficulty was finding equivalents for situations that do not arise in Poland. For example, as part of the assessment of their intellectual flexibility, American respondents were asked to answer the question: "Suppose you wanted to open a hamburger stand and there were two locations available. What questions would you consider, in deciding which of the two locations offers a better business opportunity?" In Poland there are no hamburger stands, but certain types of newsstands (kiosks) are very popular. However, the concept of entrepreneurial gain is not applicable. The final version of the Polish question was: "Suppose for a moment that you have to decide where a newsstand should be located in a new apartment development. There are two possible locations and you can choose one of them. What questions would you consider in deciding which of the two locations is better?". For measuring intellectual flexibility, the Polish version of the question seems to serve its purpose as well as the American version.

In further work, several versions of translation of each question were prepared by graduate students of the English Philology Department at the University of Warsaw. The semantic and syntactic adequacy to the English original and the effective expression of the American researchers' intent were the criteria used by the panel of experts in selecting the best Polish formulation.

The last version of the questionnaire thus obtained was subjected to a pilot study. This study was conducted by interviewing fifty persons selected from extreme groups with respect to education and type of work.

The interviewers were experienced research workers, trained to perform the interview and, simultaneously, to take notes on the reception of each question by the respondent. Subsequently, each question was evaluated with respect to the respondents' understanding of the question and the formal correctness of their answers. Then the interview schedule was reformulated and subjected to further pretests.

After the full-scale survey had been conducted and data had been coded and edited, Kazimierz Slomczynski brought a computer tape of the coded data to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Much of the work thus far has been devoted to problems of cross-national comparability of indices.

To assess whether the Polish survey had successfully replicated the earlier American survey, Slomczynski, Miller, and Kohn examined the distribution of responses for all the important questions. There were two main concerns: that each question differentiate respondents in the Polish survey, and that the patterns of these responses (or the shape of the distribution) be similar to the one expected. This examination of the central questions used in the survey showed that the questions -- both those identical to and those equivalent to the American items -- differentiated respondents, that the scales imposed on responses were suitable for the Polish population, and that the distribution of responses generally conformed to expectations. This preliminary assessment increased confidence in the comparability of measurement in the replication study, but did not in itself guarantee that these questions were capturing similar concepts.

The analysis of conceptual comparability focused on the measurement of psychological variables, particularly a battery of 57 questions about social orientation and self-conception that were asked in the U. S. and Polish surveys. Using exploratory factor analysis, the investigators found, with only a few exceptions, that each of the questions is related to the same factors in the Polish as in the American data. Using strict criteria to evaluate the adequacy of indicators in these exploratory factor analyses, the investigators then identified subsets of questions that best measured particular aspects of psychological functioning in the Polish data. Each subset of questions was then used in a confirmatory factor analysis to produce a measurement model for the concept. All such measurement models were found (by the chi-squared goodness-of-fit test) to fit the data well.

To evaluate whether these measurement models index the same concepts as those indexed in the U.S. study, another confirmatory factor model of each psychological concept was developed. These models use the questions that best index each concept in exploratory factor analysis of the American data, thereby creating models exactly parallel to those used in the original U.S. study. In every instance, the model tailored to be exactly parallel to the American model fits the Polish data well. Moreover, and of crucial importance, the correlation between the two indices of each concept is in every instance very high -- generally, in the high 0.90′s. The evidence is thus considerable that the concepts measured in the Polish replication are highly similar to those measured in the American study.

Using the indices developed by these procedures, the investigators have also carried out preliminary analyses addressed to the central questions of the Polish replication: Is the relationship between people′s position in the system of social stratification and their values and orientation similar in socialist Poland and in the capitalist U.S.? If so, is the relationship between stratificational position and values and orientation substantially attributable, in Poland as in the U.S., to occupational conditions, in particular, to the substantive complexity of work, routinization, and closeness of supervision? Tentatively, on the basis of excellent indices but only incomplete and exploratory analyses, the answer to both questions seems to be "yes." It would seem that social stratification has much the same psychological impact in the two social systems, and for much the same reasons. But, again, these findings are tentative; much analysis remains to be done before they can be accepted as definitive.

OCCUPATIONAL EXPERIENCE AND PSYCHOLOGICAL FUNCTIONING

The principal goal of this research is to assess the reciprocal relationships between occupational conditions and psychological functioning. The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler′s specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler′s specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study is to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators hope to assess with some precision the magnitudes of the reciprocal effects of occupational conditions and various facets of psychological functioning. The study of wives is designed, in part, to ascertain whether occupational conditions affect men and women similarly. (On this, see last year′s report.) These data should also enable the investigators to assess the effects of men′s occupational experiences on their wives′ psychological functioning and of women′s occupational experiences on their husbands′ psychological functioning, in each case taking account of the individual′s own occupational experiences. The study of the children is designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

During this past year, the major research efforts have been focused on six objectives: (1) Using the longitudinal data for men, to analyze the reciprocal relationship between job conditions and psychological functioning. (2) Using the data from both the men and their wives, to begin an analysis of the relationship between occupational conditions and the use of leisure time. (3) Assessing gender differences in the occupational determinants of job satisfaction. (4) Beginning an analysis of the relationship between housework and psychological functioning. (5) Examining the relationships between the structural imperatives of jobs and their rewards. and (6) Reassessing the relationship, in the U.S. and in several other countries, between social class and parental valuation of self-direction or conformity to external authority.

1. Longitudinal analysis of the reciprocal relationship between job conditions and psychological functioning. The annual report of two years ago described Melvin Kohn and Carmi Schooler's analysis of the reciprocal effects of one pivotal facet of work (its substantive complexity) and one pivotal facet of psychological functioning (intellectual flexibility). Last year's report described the extension of this causal model to encompass not only the substantive complexity of work, but also the entire set of job conditions that the investigators had earlier found to be pertinent to psychological functioning. They have termed this set of occupational conditions "the structural imperatives of the job." In further analysis this year, Kohn and Schooler refined the measurement models for several other facets of psychological functioning -- in particular, authoritarian conservatism, anxiety, trust, self-confidence, self-deprecation, idea-conformity, fatalism, and standards of morality. They then developed causal models to assess the reciprocal effects of the structural imperatives of the job and each of these facets of psychological functioning. These models show that job conditions both affect and are affected by all facets of psychological functioning that have been studied. In general, the conditions determinative of occupational self-direction -- namely, the substantive complexity of work, routinization, and closeness of supervision -- have the most far-reaching effects; but position in the organizational structure, job pressures, and extrinsic risks and rewards also matter for psychological functioning. Now in process is the development of a more encompassing model, which includes not only the entire set of job conditions but also several facets of personality, considered simultaneously. So complex a model is exceedingly difficult to create, but substantial progress was made this year. Next year's report will presumably have more to say about this analysis.

2. Occupational conditions and the use of leisure time. The purpose of this analysis is to examine the relationship between job conditions and workers' use of their discretionary time -- time not committed to earning a paycheck or to other activities necessary for meeting the basic physical

needs of the family. This research focuses on off-the-job behavior rather than on subjective psychological processes. The hypothesis is that job experiences affect and are affected by what people actually do with their time off the job, just as job experiences affect and are affected by what people feel, believe, and value.

One of the main purposes of this inquiry -- being conducted by Karen Miller and Melvin Kohn -- is to investigate the mechanism of transfer between these two spheres of life, work and leisure. A major issue in the field, to which this research is addressed, is whether workers generalize from or compensate for the rewards and deprivations of work in their behavior off the job. It is of course possible that the processes are more complicated than is implied by either a direct generalization or compensation model.

Leisure-time activities can be thought of as having a number of dimensions, just as do work conditions and psychological processes. One pivotal dimension is the extent to which people's leisure-time activity place demands on their intellectual resources. Other major dimensions of leisure-time activity are how physically sedentary or active, how solitary or social, how varied or non-varied, and how competitive or non-competitive it is. The investigators plan to test the generalization vs. compensatory hypotheses by relating occupational conditions to these several dimensions of leisure time. For example, if people directly generalize between job and non-job activities, substantively complex work should be positively related to the intellectually demanding use of leisure time, but if people compensate, then substantively complex work should be inversely related to intellectually demanding leisure-time pursuits. The investigators will also ask: do people who do physically heavy work compensate by being sedentary off the job, or do they generalize by choosing physically active pursuits? Does routine work relate to varied or to non-varied leisure-time activity? Does teamwork on the job relate to social or to solitary leisure-time activities? Do work situations requiring competition relate to competitive or to non-competitive activities outside the work setting?

As a necessary step in answering these questions, Miller and Kohn are building measurement models of the major dimensions of leisure-time activity. They have focused first on the intellectual demandingness of leisure-time activities, developing measurement models of this concept for both men and women. The indicators are hours spent watching television, frequency of attendance at plays, concerts, and museums, number of books read, time spent on hobbies, intellectual level of magazines read, and intellectual level of newspaper reading. The indicators that dominate the concept differ for employed men and employed women. For women, the time-linked indicators are the most important; for men, this is not the case. The most plausible explanation for this sex difference is the greater variation for women than for men in hours worked and time required for household responsibilities.

The measurement models for the intellectual demandingness of leisure time have provided the basis for causal models assessing the reciprocal relationships between occupational conditions and intellectual leisure-time activity. The assessment of effects can be made with more confidence for the men than for the women, because the men's data are longitudinal. For the men, almost all effects are contemporaneous rather than lagged. The effect of the substantive complexity of work on the intellectual demandingness of leisure-time activities is by far the strongest in the entire model. Job protections and income have small positive effects on the intellectual content of leisure-time activity, and hours worked has a small negative effect. In the reverse causal direction, the intellectual demandingness of leisure-time use affects only the substantive complexity of one's job. Thus, those who engage in intellectually demanding leisure-time activities mold their jobs to be more substantively complex. This may appear to be inconsistent with an earlier finding that intellectual flexibility, as assessed in the interview situation, has a lagged but not a contemporaneous effect on the substantive complexity of work. The reason for this difference may be that intellectual functioning in the interview situation measures a potential for intellectual activity, whereas the intellectual demandingness of leisure-time activities reflects a style of activity. People who seek out ways to be intellectually active in their off-the-job activities may also seek out ways to be intellectually active on the job.

Miller and Kohn's main conclusion for the men is that the substantive complexity of work is the strongest determinant of the intellectual demandingness of their use of leisure time. The causal analysis for the women is in a preliminary stage, but it appears that substantive complexity may be an important determinant of the intellectual demandingness of women's use of leisure time, too. These results provide support for the direct generalization hypothesis over the compensation hypothesis. It remains to be seen whether this same learning model holds for other dimensions of off-the-job behavior, to be explored in the next phase of the analysis.

3. Gender differences in the occupational determinants of job satisfaction. Joanne Miller's analysis focuses on gender differences in job satisfaction. Men's and women's orientations to work are said to differ in fundamental respects. Differential socialization before entry into the labor force, dissimilar parental and conjugal roles, and systematic differences in occupational opportunities suggest that the meaning ascribed to work and rewards sought may vary by sex. The same job conditions may be more or less central for job satisfaction because male and female workers differ in their orientation to work.

Job satisfaction is defined as the cumulative subjective evaluation of work experiences. As such, the content of job satisfaction can vary by the importance attached to particular features of the job. Nineteen specific criteria for judging work were included in the occupational surveys. In an effort to capture both the content and intensity of judgments made about

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work, the investigators asked respondents about their degree of satisfaction (or dissatisfaction) with the specific aspects of their jobs they deemed important. Job satisfaction is operationalized as the average of these judgments, weighting a person's satisfaction with an aspect of work he deems very important twice as much as his satisfaction with an aspect of work he considers only fairly important. This approach assumes that a job is not evaluated as a single entity but as a set of multi-dimensional tasks performed in a particular physical and social context.

Focusing on possible gender differences in the content of occupational satisfaction, Miller asked which aspects of work are most important to men and which to women. Both men and women consider a broad range of job characteristics important. Overall, men and women seem to judge work along similar dimensions -- especially in terms of economic advantage, interest, pride, and relationships with other people in the immediate work setting. The sex of a worker is not systematically related to the importance placed on pay, fringe benefits, how interesting the work is, the chance for exciting work, the supervisor, co-workers, opportunity to feel pride in craftsmanship, or job security. Nevertheless, there are some systematic sex differences in work values. Men are more likely to emphasize positional authority--the opportunity to exercise leadership, make decisions, and get ahead--while women are more likely to find the chance to help people an important reward. Another gender difference in judging work involves the convenience aspects of the job. Hours, cleanliness, how tiring the work is, and job pressure are of more concern to women than to men.

To the extent that women's criteria for judging work are systematically different from men's, one might expect gender differences in the determinants of job satisfaction. But, even if men's and women's orientations to work were identical, adjustment to job conditions could still be contingent on gender. In this part of the analysis, Miller examined how various aspects of work are related to job satisfaction, focusing on those job conditions that are most central to the nature of the work performed. These include job conditions that determine the level of occupational self-direction, job pressures, organizational structure and position, job uncertainties, and rewards and protections of the work. The analysis shows a strong relationship between day-to-day job experiences and job satisfaction, regardless of the worker's gender. It is of course possible that socially selective recruitment into and retention in particular occupations might explain these relationships between job conditions and job satisfaction. It is also possible that there is selection into jobs on the basis of a psychological predisposition to be satisfied or dissatisfied. Miller's analysis therefore statistically controls earlier job satisfaction and background characteristics. Even then, there is a strong relationship between job conditions and job satisfaction for both men and women. Intrinsic aspects of work are stronger determinants of job satisfaction than either the extrinsic rewards of the job (income and job protections), job uncertainties, or the background of respondents, for both men and women.

Although both men and women evaluate their jobs in terms of the work performed, there are differences in the particular job conditions that influence their assessments. For women, the substantive complexity of work, dirtiness, and number of hours worked are more pertinent to job satisfaction, while for men closeness of supervision and position in the organizational hierarchy are more pertinent. Time pressure is important for both men and women. Miller also demonstrated, in reciprocal causal models, that the significant effects of job conditions on job satisfaction cannot be attributed to job molding -- i.e., to satisfied employees carrying out their work differently from dissatisfied workers. These findings confirm that day-to-day job conditions determine job satisfaction and that occupational self-direction is an important criterion for judging work, regardless of the sex of the worker.

4. Housework and psychological functioning. On the hypothesis that the psychological impact of the conditions of work encountered in household work would be similar to the impact of work conditions encountered in paid employment outside the house, Kohn and Schooler included in the 1974 follow-up survey a set of questions about the nature of the actual work performed by the respondent in taking care of the household. Insofar as possible, these questions exactly parallel those asked about work performed in paid employment. The household-work questions were asked not only of housewives, but also of working wives and of men.

With this information, Carmi Schooler, Melvin Kohn, Joanne Miller, and Karen Miller have developed measurement models of the basic conditions of work encountered in housework. Using confirmatory factor analysis, they have developed models of: time spent on housework, the substantive complexity of household work, closeness of supervision (by one's spouse), and routinization. The relationships between concepts and specific indicators are different for men and women; in general, these differences parallel the traditional sexual division in housework responsibilities. Thus, the substantive complexity of the work men do around the house is strongly characterized by the complexity of the repairs they make, while the substantive complexity of women's housework is characterized by the complexity of a wide variety of tasks they perform, one of the most important being the complexity of their sewing.

Having created these indices, the investigators are now developing causal models of the relationships between housework and psychological functioning. The initial analyses have been limited to women; they have examined the relationship between women's housework and their intellectual flexibility. The results thus far indicate that women's housework has a profound effect on their intellectual flexibility, the substantive complexity of housework and the closeness with which it is supervised having even greater effects on intellectual flexibility than do the parallel measures of work conditions in paid employment. These results are preliminary and tentative. Schooler and his colleagues intend to analyze the data for housewives and for working women separately, to assess the psychological effects of housework on men, and to take into account the effects of occupational conditions encountered in paid employment, which have thus far not been statistically controlled. Still, the strength of the initial findings makes it seem probable that the main hypothesis -- that the conditions of work encountered in housework will have psychological effects similar to those of the conditions of work encountered in paid employment -- will be confirmed.

5. The structural imperatives of jobs and their rewards. The basic concern of this facet of Carmi Schooler's research is the nature of the relationship between work and its rewards. Specifically, he asked how the structural imperatives of men's jobs are related to both the objective and subjective rewards that jobs provide. The specific rewards considered here are the job's social standing, the income it provides, the incumbent's assessment of its prestige, his sense of the prestige it is accorded by others, and his subjective social class identification.

Schooler found that the substantive complexity of work is by far the most important determinant of the job's social standing. As a determinant of income, though, substantive complexity is less important; in this case, ownership, absence of close supervision, and a high position in the supervisory hierarchy have notable effects.

The importance of ownership, hierarchical position, and closeness of supervision as determinants of income is congruent with interpretations that stress the importance of control over the means of production, both for the distribution of occupational rewards and also in the formation of social classes. This raises the question whether personal perceptions of one's occupational status similarly are affected by the individual's relationship to the control of production. Relevant data exist in the 1964 survey, where respondents were asked to identify the social class to which they thought they belonged and to rank the prestige of their occupations both in terms of the way they themselves evaluate them and the way they believe others do. Of the three structural imperatives of a job linked to control of production -- ownership, hierarchical position, and closeness of supervision -- the most important determinant of the individual's view of his social position is closeness of supervision. However, when all of the structural imperatives of the job are included in the regression equations, the substantive complexity of work is by far the most important determinant of the individual's view of his prestige and of his position in the class system.

Several theorists have suggested that there might be other categorizations of occupational status more powerful than the linear measures of occupational status usually employed in stratification research. Among the criteria of categorization that have been suggested are: white-collar vs. blue-collar; manual vs. non-manual; a "higher" social class that includes skilled workers vs. a "lower" social class of semi-skilled and unskilled workers (embourgeoisement of the working class); a "higher" social class of professionals, managers, and the like vs. a "lower" social class that includes clerical and lower white-collar workers (proletarianization of the lower middle-class). It is possible to test the effect of these several categorizations by seeing whether including any of them in regression equations for income or subjective class identification either adds to the total explained variance or decreases the regression coefficient for the linear measure of occupational status.

Of the various non-linear criteria for categorizing workers into social classes, only manual vs. non-manual and blue-collar vs. white-colla (measured literally by asking respondents what type of clothing they wear on the job) have significant independent effects on the individual's conception of his job's prestige and his social-class position, and even these effects are much smaller than those of the linear measure of occupational status. In terms of affecting income, only working with things has a significant independent effect when included in the regression equation together with occupational status. In short, the usual method of treating occupational status as a linear variable accounts for the lion's share of the variation in the subjective and objective rewards that jobs provide.

6. Social class and parental valuation of self-direction or conformity to external authority. Kohn and Schooler's factor analysis of the data on parental values in the 1964 survey found the first dimension to be valuation of self-direction as contrasted to conformity to external authority: the characteristics that are positively correlated with valuing self-direction are an interest in how and why things happen (i.e., curiosity), consideration, self-control, good sense and sound judgment, and responsibility; the characteristics that are positively correlated with valuing conformity are neatness and cleanliness, good manners, being a good student, and obedience to parents. Th samee parental-values questions have subsequently been included in several other U.S. studies, notably the National Opinion Research Center's General Social Surveys of 1973, 1975, and 1976. Melvin Kohn and Carrie Schoenbach did factor analyses of fathers' and mothers' values for each of the three NORC surveys, in each case asking whether the first unrotated factor (i.e., a single-factor solution) reflects self-direction/conformity to external authority. Separate analyses of fathers and mothers in all three years confirm unequivocally that it does.

One might wonder whether this finding somehow reflects a particularly American concern with self or individuality or independence. Kohn hypothesized, to the contrary, that self-direction/conformity to external authority is an invariant dimension in parental values in all industrialized societies. Data with which to test this hypothesis have generously been provided by colleagues who have done studies of parental values, modelled on the Kohn-Schooler inquiry, in six disparate countries: Italy, West Germany, Taiwan, Ireland, Poland, and Japan. Kohn's hypothesis -- a very strong hypothesis -- was that the first unrotated factor in all these bodies of data would be a clear self-direction/conformity dimension. In general, this hypothesis was confirmed, although in two studies (one done in Ireland, and one of the two done in Japan) he had to go to a two-factor rotated solution to find a clear self-direction/conformity dimension. Still, the principal finding is a striking affirmation of the presence of a self-direction/conformity dimension in parental values in all the countries studied.

The correlation between social class and fathers' valuation of self-direction, in the original Kohn-Schooler study, is 0.34, and it is of roughly similar magnitude in subsequent studies of both fathers' and mothers' values. This correlation is rather substantial, considering that correlations between any single dimension of social structure and psychological phenomena never are very large. After all, there are many additional determinants of values and orientation -- idiosyncratic personal experience, situational constraints, cultural and institutional imperatives, and facets of social structure other than class itself. Still, Kohn and Schooler place such great theoretical weight on values as the bridge between social structure and behavior, that it is desirable to reassess the magnitude of the relationship between social class and parental valuation of self-direction or conformity to external authority, using newer methods of analysis than were available at the time of the original study. With confirmatory factor analysis, Kohn, Schooler, Schoenbach, and Karen Miller have developed measurement models of parental values for three studies based on national (as distinct from single-city) samples -- the 1964 Kohn-Schooler study, the NORC General Social Surveys, and the Polish replication. These models show the class-values correlation to be approximately 0.45 - 0.50 for both mothers and fathers. With the longitudinal data of the Kohn-Schooler follow-up study, which permits a much more precise measurement model of parental values, the correlation between social class and fathers' valuation of self-direction/conformity is approximately 0.60. This is the most accurate estimate of the actual magnitude of the correlation possible with present methods. It is indeed impressive.

THE EFFECTS OF ADULT LIFE CONDITIONS ON SEX-ROLE IDEOLOGIES OF FACTORY WORKERS IN INDUSTRIALIZING SOCIETIES

Karen Miller's study of the effects of industrialization on family and sex-role ideologies of factory workers is now completed. In the portion of the analysis completed last year, she analyzed sex-role ideology as a product of industrialization in one nation, Bangladesh. Her tentative conclusion was that two occupational conditions -- the skill level of the job and benefits provided to the worker by the factory -- were the aspects of an individual's experience with industrialization that were most related to the belief that women should have equal rights. This year she extended the analysis to several cultures, and broadened the focus to include ideologies about family relationships as well as about relationships between the sexes.

The data used in the analysis consist of interview responses from about 3800 male factory workers in Argentina, Chile, Bangladesh, India, Israel, and Nigeria. The data were obtained in 1964/65 by the Project on Social and Cultural Aspects of Development, directed by Professor Alex Inkeles, now at Stanford University.

Confirmatory factor analyses yielded acceptable measurement models of an ideology about autonomy of the adult from parents and an ideology about extrafamilial rights of women in India, Bangladesh, aFd Israel, but not in Argentina, Chile, and Nigeria. These results indicate that there is some, but not universal, cross-cultural regularity in the content of family and women's-rights ideologies. The correlations between the two ideologies a low to moderate; thus Miller concluded that they should be treated separately. Path analyses with each ideology as the dependent variable showed differences in the direct effects of variables related to industrialization. Mass-media exposure relates negatively to family ideology but positively to women's-rights ideology; education and economic circumstances are important for family but not for women's-rights beliefs; job skill level relates to women's-rights but not to family beliefs. When Miller takes into account indirect as well as direct effects, however, a similar set of variables comes into play for both ideologies. Age and father's education, respondent's education and intellectual skills acquired, job skill level, living standard, and mass-media exposure all have some effect, either direct or indirect, on both family and women's-rights ideologies. Thus, many aspects of industrialization apparently play a part in shaping egalitarian attitudes.

SOCIAL-STRUCTURAL SOURCES OF STRESS

In another major study, Leonard Pearlin has been examining the relationship between social experience and psychological well-being. His research looks at the enduring problems people encounter in their daily lives as workers and family members, at the gain and loss of roles as the life-cycle unfolds, and at sudden, unexpected crises that often confront people. Each of these types of experience is examined in relation to symptoms of emotional distress. Data for the study were collected at two times. In 1972 a sample of 2300 people representative of the Chicago Urbanized Area were interviewed. These interviews sought information abou three features of people's lives: the chronic, often low-keyed, everyday problems that people encounter in their occupational, marital and parental roles; the coping strategies people employ in dealing with life-problems; and symptoms of emotional stress, anxiety and depression. Then in 1976-77 a second wave of interviews was conducted among a sub-sample of 1200 peopl drawn from those originally interviewed. Many of the questions that had initially been asked about chronic role problems, coping, and psychologica distress were repeated, thus providing longitudinal information about stability and change in these aspects of people's lives. Additional questions sought information about key life-events that occurred since 1972, both the many changes and role transitions that are normative aspect of the life-cycle and the more unexpected, non-normative disruptions of important roles and activities, such as divorce, serious illness, involuntary unemployment, and other life crises.

The two sets of interviews provide a large body of information about problems woven into the fabric of major social roles, the gains and losses involved in life-cycle transitions, the crises that intrude into people's lives, coping repertoires and psychological outcomes. It is the long-range goal of the research to reconstruct from this information those elements of social experience that importantly affect mental health and to learn how people attempt to avoid being psychologically harmed by such experience.

During the past year, Pearlin's work proceeded along both methodological and substantive lines. The major methodological task has been the development and refinement of all of the measures for which he has longitudinal data. He has completed this phase of his work, enabling him to evaluate life-changes with a high level of reliability.

The substantive efforts were directed primarily at further explorations of the conditions that people experience at different phases of the life-course. There is an established view that family transitions, in particular, represent important turning points in people's lives. These transitions include such key events as getting married, having children, seeing children move through various stages of development, the launching of children from the household, becoming a grandparent, and being widowed. It is frequently assumed that the major reorganization of life demanded by such events and transitions may result in severe emotional distress. Contrary to these expectations, however, Pearlin found that family transitions--with the exception of being widowed--have virtually no effect on psychological functioning. One apparent reason for their lack of impact is their predictability. By being so closely linked to the life-cycle, transitional events are typically forecast in advance of their occurrence. Much of the adjustment to these events, therefore, may take place anticipatorily, thus minimizing the effects of the actual event.

In contrast to family transitions, certain life-changes that are less easily forecast do exert an emotionally disruptive effect. Outstanding in this respect are the following: involuntary loss of a job, especially for reasons of poor health; divorce and separation; the death or serious illness of a child; and, as earlier noted, being widowed. When allowances are made for prior emotional states and when effects of major social characteristics are statistically controlled, these are the events that stand out as being most potent. These particularly powerful events notwithstanding, people generally adapt to change with considerably more ease than might be expected from theories of stress that emphasize the importance of equilibrium to psychological well-being.

Pearlin has also continued to test certain assumptions and practices found in studies of the aging process. One such assumption, more implicit than explicit, is that aging is inherently difficult. Both the accumulation of unwanted changes through time and the inevitable approach of death would seem to make aging an intrinsically stressful process. However, it is not; in fact, stress generally declines with age, at least until the time that people become infirm. The challenges and problems of assuming new roles make the earlier years of adulthood more stressful than the later years.

These findings point up the methodological advantage of making comparisons across different age ranks rather than following the more common strategy of studying single age groups separately. Comparative perspectives are essential to discovering the circumstances that are distinctive of a particular life-stage and those that are not. In addition, it is imperative to make comparisons within as well as across age groups. It is a repeated finding that being of the same age does not override the social and economic differences among people. That is, people belonging to a cohort do not necessarily experience similar conditions of life because they are the same age. Instead, their experiences depend on their social and economic characteristics. It is indefensible, therefore, to speak of age groups as though they have traversed a uniform life-course.

Annual Report for 1979

Laboratory of Brain Evolution and Behavior

National Institute of Mental Health

Paul D. MacLean, M.D., Chief

Recently a remarkable skull of a monkey found in Patagonia was described. Identified as Dolichocebus gaimanesis, it is dated as belonging to the Oligocene epoch. According to the author of the report, "The specimen strongly suggests that Dolichocebus is a member of the Saimiri lineage, which thus becomes the oldest generic lineage known for primates, dating from about 25 million years ago." It is an awe-inspiring experience to superimpose the skull of a present-day squirrel monkey over the one of Dolichocebus and note the close overlap not only of the cranium, but also of the teeth, right down to the slightly buck-toothed appearance of the incisors. The comparison indicates that morphologically, the squirrel monkey has changed very little over a period amounting to 8 million generations. The fossil in question is of enhanced interest because of recent evidence suggesting that in locomotion and other respects Aegyptopithecus and Dryopithecus (two pivotal forms in the evolution of primates) may have been more like that of living New World Ceboids than Old World monkeys.

In the light of the foregoing considerations, it seems a fortuitous circumstance that the squirrel monkey has been a focal experimental animal in our Laboratory for over 20 years. In addition to anatomico-physiologic studies on vision and cerebral representation of sexual functions, they have been used extensively for investigations on cerebral mechanisms underlying species-typical, prosematic (nonverbal) behavior, about which more will be said after a few explanatory statements.

The R-complex and species-typical behavior. The neurobehavioral work of our Laboratory is primarily concerned with investigations on the anatomy and functions of the striatal complex, a basic part of the mammalian forebrain that represents an evolutionary heritage from reptiles. The striatal complex (hereafter foreshortened to R-complex) consists of the olfactostriatum, corpus striatum (caudate nucleus and putamen), globus pallidus, and satellite collections of gray matter. Contrary to the traditional clinical view, the R-complex does not appear to be primarily motor in function. In our neurobehavioral experiments, we are testing the hypothesis that the R-complex is basically implicated in the organized expression of species-typical, prosematic behavior. In animal communication four main types of displays are used for conveying information. They may be identified as (1) signature displays; (2) "distant" and "near" challenge displays; (3) courtship displays; and (4) submissive displays. Since 1961 we have conducted experiments on the effects of various cerebral lesions on the species-typical mirror display of "gothic-type" squirrel monkeys. The mirror display is a highly predictable variation of a naturally occurring display used by male squirrel monkeys in a show of aggression, in courtship, and in greeting. In earlier work it was shown that certain cascading projections from the R-complex were basically implicated in the display. In this year's experiments, evidence was obtained that ascending connections to the R-complex are also involved in the display. These ascending connections appear to run in the central tegmental tract, one of the largest and most ancient pathways of the brainstem.

In addition to prosematic communication, it is also hypothesized that the R-complex is basically involved in organizing activities that constitute an animal's daily master routine and subroutines. In preceding work it was shown in hamsters deprived of neocortex since birth, that the R-complex and limbic system are sufficient for giving expression to the recognized forms of species-typical behavior. In the work this year it was found that with additional loss of the midline limbic cortex, play-fighting may not occur, and there may be deficiencies in maternal care of the young. These results might have been expected on the basis of known differences in the behavior and brain structure of reptiles and mammals.

The R-complex and endorphins. The question of the function of the endorphins is relevant to the work of our Laboratory because of their known high concentration in parts of the R-complex and in certain structures of the limbic system. It was found in hamsters that there was a highly significant increase in serum endorphins after a copulatory period involving five ejaculations. Another series of experiments revealed that the administration of naltrexone (believed to compete with morphine-like drugs at opiate receptors) resulted in increased potency. This heretofore unrecognized effect of the drug suggests that clinically it might have some application in the treatment of impotence.

The R-complex and iron. In the neuronatomical laboratory an improved method has been devised for demonstrating the presence of iron in the brains of lower animals. Comparative studies have shown that (as long known in the case of the human brain) iron is found in particularly high concentration in the perineural glia of the globus pallidus. In addition, it has been found in similarly high concentrations in specific parts of the olfactostriatum in opossums, rats, hamsters, and squirrel monkeys. The area involved deserves the designation paleopallidum. In rats, the condition of pregnancy or the administration of estrogen to spayed females results in a marked increase of pallidal iron. The significance of this and related discoveries, as well as metabolic implications in regard to acetylcholine, dopamine, and GABA, is to be further investigated.

The R-complex and the epiphysis. Reflecting an ancient relationship, indirect neural connections between the R-complex and the epiphysis via the habenula exist in some animals. In its entirety the extracerebral epiphysis includes the pineal body and parietal eye (third eye). The connected parietal eye and pineal body of living reptiles constitutes the primitive pattern for all vertebrates. In the advanced forms of mammal-like reptiles that were ancestral to mammals, there was a disappearance of the parietal eye.

The workers in the Paleoneurology Wing are following up their original findings that destruction of the parietal eye or the pineal body leads to an alteration in temperature regulation that subsequently has a retardant effect on reproduction. Parietalectomy retards pre-vitellogenic ovarian follicular growth in the early phases of ovarian recrudescence, but retards growth in the later phases only if the animals are prevented from freely regulating their body temperature.

Most interestingly, melatonin has been demonstrated to be present in very small amounts (125 pg/ml) in the serum of alligators throughout the light-dark cycle. These animals, which are nocturnal, lack both the pineal body and parietal eye, indicating an extrapineal source of this hormone.

Breath-related neurons. Last year we reported that about 10 per cent of neurons encountered in the three main evolutionary formations of the forebrain discharge in association with respiration. This discovery has profound implications with respect to the possible role of such neurons in states of wakefulness and sleep, as well as in many psychological states affected by respiration. This past year's work has shown that the discharge of such units in the insula, amygdala, hippocampus, and elsewhere is not dependent on the olfactory nerves.

The Switzer-Merril method for detecting proteins and peptides. Finally, special credit is owing to the comparative neuroanatomist in our Laboratory for recognizing the potential power of the de Olmos silver stain in connection with the two-dimensional gel technique employed for detecting proteins and peptides. Heretofore, it has not been possible to realize the power of the two-dimensional gel technique of O'Farrell with conventional stains. Rather, it has been necessary to employ time consuming expensive radioassays to obtain the desired sensitivity. Because of dangers or prohibitive expense, the radioassays cannot be applied for certain essential purposes in animals and human beings. The present Switzer-Merril method is 100 times more sensitive than the commonly used Coomassie blue stain and may equal or surpass the radioassay method. It provides a relatively quick, simple, inexpensive, and harmless method for detecting protein and peptide patterns in a variety of tissues. It provides, for example, a ready means for surveying protein patterns in the blood, urine, and spinal fluid (without need of concentration) of patients with diagnoses of schizophrenia or manic-depressive psychosis. Other possible applications are too numerous to mention. In our Laboratory we plan to use it for identifying protein and peptide patterns in various structures of the central nervous system.

UNIT FOR RESEARCH ON BEHAVIORAL SYSTEMS

The staff of the Unit for Research on Behavioral Systems (URBS) have had a productive year in their continued effort to identify genetic, ecologic, and behavioral factors that may either lead to or prevent a breakdown of social organization under overcrowded conditions. A particularly important aspect of this work is to discover how the design of experimental habitats affects social organization and survival. Given 20 years as an average survival time of most human modern dwellings, it is predicted that 200 million housing units will be required each year for the next 80 years, making a total of 16 billion by 2065. Such calculations lead one to realize that "no single area of intellectual effort can exert a greater influence on human welfare than that contributing to better design of the built environment."* The physical habitat, so to speak, constitutes the anatomy of any human or animal society.

Habitat design: Findings on mice. Through the redesign of a large habitat used in an earlier study of crowding in mice, it has been discovered that "in the maturation of communities comprised of several social groups, each with its space specific activities, both the activities within a group's range and the orientation of groups to each other, gradually shift from a radially symmetrical arrangement to a bilaterally symmetrical one." Most significantly it has been found that the design of the present habitat has apparently allowed the optimum number of mice to become more than double the theoretically predicted value.

Influence of co-operative behavior on population growth of rats. The above study on mice has revealed how improved design of a habitat allows a greater number of animals to occupy a given area of space than would be expected. Another ongoing study of a growing population of rats focuses particularly on the influence of co-operative activity with respect to tolerance of more than a usual number of animals in a well designed habitat. In the experimental population, opportunities are provided by means of a computer-controlled system for certain assigned animals to co-operate, for example, in obtaining water, while in the control group no such possibilities exist. Another aim of this study is to learn whether or not the co-operative situation will encourage variations in vocalization for the purpose of communication. A sophisticated recording system and computer-assisted analysis make possible the detection of new vocalizations or differences in the number of the same kind of vocalizations. Another year will be required before the present project will begin to yield the required data, but in the meantime there has been an interesting ancillary finding. It has turned out that the special kind of rat bred for this study by crossing two domesticated and two wild strains is a kind of "super rat." Not to be confused with Nietzsche's superman, it shows a kind of intelligence and an interest in human beings not seen in ordinary rats. It also makes more complex nests than the usual rat.

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*This and subsequent quotations from separate summarizing report by John B. Calhoun.

An evolutionary world population model. An analysis of human population growth has indicated that by 1975 there began a world-as-a-whole decline in human fertility. Based on present trends it appears that that the world population will reach a maximum of nine billion in 2065. Thereafter the population will begin to decline. The maximum mean age will be reached in 2080, with 58 per cent of the people over 40 years of age. At that time only about 10 per cent of the total population is expected to be involved in the production of children.

As the URBS investigators have stated, "A value of our theory is that it depicts the character of the transition and how it forms a natural phase of the evolutionary process. It is the only existing nonnegative model; all others are based solely on adjustment to dwindling natural resources. Our theory proceeds from the thesis that the 'arrow' of evolution provides survival value to forms of life capable of shifting the entropy balance toward increased amounts of diversity of information along with greater information processing capacity per unit of living matter.... The next phase shift in population can only be accomplished by a reduction in the human population within which individuals develop stronger bonds with even more sophisticated information processing machines which assume more characteristics of living matter."

It would seem that this model would satisfy Aristotle's optimistic philosophy of striving, with life forces ever spiraling upwards from one potential to another. A ceaseless striving to achieve our human potential!

Annual Report of the Laboratory of Cerebral Metabolism, NIMH

Louis Sokoloff, Chief

October 1, 1978 through September 30, 1979

The Laboratory of Cerebral Metabolism has continued to operate as two sections, the Section on Developmental Neurochemistry, and the Section on Myelin Chemistry. These two Sections maintain their own distinct and independent research programs, but both make common use of many of the facilities, equipment, and personnel.

The Laboratory has been suffering acutely from the consequences of its success. Its research during the last few years has led to the development of the deoxyglucose method for the measurement of local cerebral glucose utilization and the demonstration that local glucose utilization is coupled to local functional activity and can be used to identify minute regions of the central nervous system with altered functional activity in physiological, pharmacological and pathological states. This research has received widespread recognition which has transformed the Laboratory into a world research center with all the demands, responsibilities, and burdens associated with this status. Because the methods developed in this Laboratory are useful in almost every subdivision of neuroscience, there have been world-wide demands on the Laboratory to educate and train other investigators in their use and application. This requires not only a considerable amount of consultation, lecturing, and travel, but also collaborations and the acceptance of investigators from all over the world for such training, usually supported by their own organizations. In some cases we have accepted Visiting Fellows, Associates, and Scientists, when we thought that they would contribute substantially to our own research program.

The development of these methods has also expanded enormously the scope of the research of the Laboratory. Our own use of these methods has forced us to extend our activities from previously only neurochemistry to include also neurophysiology, neuropsychopharmacology, neuropathology, and neuropsychology. As with all successful new methods that make it possible to do things never done before, new findings and new directions are uncovered almost every week. The result of this success has been enormous strain on the resources of the Laboratory.

The resources allocated to this Laboratory have never been optimal, but they are now totally inadequate to cope with the present needs. The secretarial staff is completely overloaded and cannot keep up with the load of correspondence, memoranda, and manuscript preparation. Maintenance of adequate files is impossible because of lack of filing capacity or even of space in which to place additional file cabinets. Current research records, even though active, are frequently stored in cardboard boxes and placed in any open spot available. Shelves throughout the Laboratory are filled to overflowing. Population density in the rooms is excessive; there are in some cases as many as three individuals in a half module. Even the Laboratory Chief does not have a private office; his office serves also as a library and computer room. Every nook and cranny of every room is packed to overflowing. With such a shortage of space, it is impossible to utilize space on any rational functional basis; allocation must generally be made on the basis of improvisation and temporary expediency. Indeed, the current problems of space are often more difficult to solve than the scientific problems.

Nevertheless, despite the almost inhumane physical working conditions, the Laboratory continues to make significant progress in its research.

Section on Developmental Neurochemistry

Louis Sokoloff, Chief

The major activities of this Section have been in the applications of the deoxyglucose method in neurophysiology, neuropharmacology, neuropsychology, and neuropathology. The studies by C. Kennedy and others on mapping the primate binocular primary visual system have been completed. In collaboration with M. Mishkin and C. Jarvis of the Laboratory of Neuropsychology, NIMH, he is presently applying the method to trace visual pathways in the monkey beyond the striate cortex. They have already found surprisingly broad involvement of the inferior temporal cortex in higher visual functions.

M. Miyaoka has been studying the visual system of albino and pigmented rats. He has found that in both strains local glucose utilization in the primary projection areas from the retina obeys the Weber-Fechner Law, indicating that local metabolic activity is quantitatively related to behavior. The visual cortex, although activated by retinal stimulation, did not obey the Law, perhaps because it is more involved in processing input rather than responding directly to it.

P. Yarowsky has been studying the relationship between electrical and metabolic activities. For these studies he is using the superior cervical ganglion of the rat in which the preganglionic fibers can be isolated and electrically stimulated. Thus far, the results indicate a close quantitative relationship between the spike frequency and glucose utilization in the normal physiological range of frequencies.

M. Kadekaro and H. Savaki have used the method to map the central neural pathway in the gastrosecretory response to hypoglycemia.

The studies on the effects of amphetamine by L. Wechsler and H. Savaki have been completed. These studies were the first to demonstrate the effectiveness of the deoxyglucose method in neuropharmacological studies. They demonstrated effects in the entire extrapyramidal system, including unexpectedly the subthalamic nucleus. Also surprising was the finding of a lack of effects in the mesolimbic system, which had been postulated as the site of action of amphetamine responsible for its psychotomimetic effects. McCulloch and Savaki have also completed studies on the effects of the dopamine agonist, apomorphine, and have found typical response-log dose activation of a variety of dopaminergic areas in the brain. The full results of these studies are still being analyzed. Savaki is also completing her studies on the effects of α- and β-adrenergic agonists and antagonists. J. McCulloch and H. Savaki, in collaboration with A. Pert and W. E. Bunney of the Adult Psychiatry Branch, NIMH, have studied the phenomenon of supersensitivity of dopaminergic synapses associated with chronic neuroleptic therapy. They have been able to identify dopaminergic structures that became supersensitive following prolonged haloperidal administration on the basis of the metabolic response to a challenge dose of apomorphine.

C. Smith and L. Davidsen have been studying the effects of normal aging on local cerebral glucose utilization in the rat. They have found extensive widespread reductions, but most prominently affected are the components of the extrapyramidal system and the primary sensory pathways, particularly visual and auditory pathways.

M. Miyaoka and C. Kennedy have studied, in collaboration with R. Post of the Adult Psychiatry Branch, NIMH, the effects of lidocaine-kindled seizures in the rat. This appears to be a model of temporal lobe epilepsy, and the results demonstrate particular involvement of the limbic system in the pathogenesis of these seizures.

M. Shinohara has completed and published her studies on the effects of spreading cortical depression on local cerebral glucose utilization. This is a question of long-standing interest, but her results have provided a final definitive answer. Spreading cortical depression markedly increases blood flow and glucose utilization in the cerebral cortex while reducing these functions in sub-cortical structures.

In addition to applications of the deoxyglucose method, the Section has been continuing methodological research. C. Goochee and W. Rasband of the Section on Technical Development, NIMH, NINCDS, have developed a computerized image-processing system for densitometric analysis of the deoxyglucose autoradiographs and their reconstruction on a TV monitor as color-coded maps of brain sections displaying the distribution of the actual rates of glucose utilization in every spot of the brain. This technique greatly enhances the resolution and sensitivity of the deoxyglucose method.

C. Smith and L. Davidsen have continued the development of a method to measure local rates of cerebral protein synthesis comparable to the deoxyglucose method that measures local glucose utilization. They have made substantial progress, and we hope to have a usable method within the next year.

Section on Myelin Chemistry

Marian W. Kies, Chief

The research of the Section on Myelin Chemistry is mainly concerned with the biochemistry and immunology of two basic proteins which are localized in central (CNS) as well as peripheral (PNS) myelin. The basic protein (BP) in CNS myelin is capable of inducing an autoimmune disease, experimental allergic encephalomyelitis (EAE). The same BP is also found in the PNS, where it is accompanied by a second less basic protein, P-2. Both proteins are considered to be structural proteins because of their relatively high concentration in myelin. They may or may not possess any specific catalytic activities.

It is known, however, that BP is an excellent substrate for *in vivo* and *in vitro* phosphorylation by protein kinases and this reactivity may have some metabolic significance. Dr. Russell Martenson, who has been on a foreign work assignment in the neurochemical laboratory of Dr. Jean-Marie Matthieu in Lausanne, Switzerland, has undertaken an in depth study of the *in vivo* phosphorylation of BP as a probe into its metabolic function. Studies on the structural correlates of its *in vitro* phosphorylation have been carried out in the laboratory here.

In continuation of a collaborative experiment with Dr. Janis Young, Mrs. Deibler has purified and subjected to bioassay several synthetic peptides representing potential encephalitogenic regions of BP. In addition, these peptides will be used to explore *in vitro* activation of BP-sensitized cells, location of specific antigenic regions in the BP molecule, and potential sites for phosphorylation.

Mrs. Bacon and Mrs. Deibler have been studying induction of experimental allergic neuritis (EAN, the PNS counterpart of EAE) in rats. Although P-2 is thought to be the specific neuritogen in PNS tissue there have been many conflicting reports. We believe that the difficulty in obtaining conclusive bioassay data lies in the inability of some investigators to distinguish between two similar diseases in the same host. In order to demonstrate that P-2 induces EAN we feel that the isolated protein must be entirely free of BP. Mrs. Deibler has continued to explore satisfactory procedures for separating BP and P-2 from peripheral nervous tissue.

Dr. Bernard Driscoll, Guest Worker supported by the National Multiple Sclerosis (MS) Society, and Dr. John Richert, MS Postdoctoral Fellow, have been using adoptive transfer of EAE in inbred guinea pig and rat strains to study mechanisms of autoimmune damage in the CNS. Dr. Driscoll discovered last year that transfer of EAE could be enhanced approximately 100-fold by *in vitro* incubation of sensitized peritoneal exudate cells (PEC) with antigen (BP). The potentially active cells are present in the peritoneal exudate 6 days after sensitization of the donors. Maximal activity is obtained after 18 hours culture with BP, 10 μg/ml. These data suggest that the critical reactio in culture is presentation of antigen by macrophage-like cells and that proliferation *in vitro* is not required. However, the fact that mitomycin C prevents both proliferation and transfer suggests that cell proliferation must occur in the recipient for transfer to be successful.

Guinea pig lymph node cells (LNCs) differ markedly from PECs in their response to BP *in vitro*. They are not stimulated to proliferate, and their ability to transfer is only slightly enhanced by BP. However, enhanced transfer is obtained if unsensitized PECs are cultured with sensitized LNC and BP. After culture, the cells can be fractionated by centrifugation on a BSA concentration gradient to yield light (dividing cells) which transfer EAE with as few as 5 x 10^6 cells. The more dense cells do not transfer EAE.

Incubation with specific antigen also leads to enhanced transfer of EAE with sensitized Lewis rat spleen cells. The two species (guinea pigs and rats) are very different in their immune response to BP--in fact, guinea pig spleen cells cannot be activated with antigen *in vitro* to transfer EAE. Although the time course and BP concentration requirements of the two systems (guinea pig PEC and rat spleen) are somewhat comparable, rat spleen cells require a longer incubation time for maximal activity and respond to lower concentrations of BP.

In both species, we are attempting to identify and characterize the specific cell type responsible for disease and to use the transfer technique to search for suppressor cells.

Annual Report of the
Laboratory of General and Comparative Biochemistry
National Institute of Mental Health

Giulio L. Cantoni, Chief

In fiscal year 1979, the Laboratory of General and Comparative Biochemistry has shown continued progress and good productivity.

The shortage of budgeted laboratory positions imposes a handicap to all the members of our senior staff, but in response to the difficulties, we have all attempted to respond immaginatively by developing fruitful collaborations with other colleagues at NIH and elsewhere. Although this development is not without its problems, it is clearly the best solution until the more fundamental question can be addressed and resolved. I commented in the Annual Reports of 1977 and 1978 on the ridiculously low travel budgets. The restrictions on mobility continues to be a very serious, indeed, an intolerably serious handicap. Contacts with our colleagues at various universities and research institutes in the United States, either through visits, seminars, meetings, workshops, etc. are an essential function for our senior scientists and an equally important activity for younger colleagues who depend on these contacts for their professional development. I know that our administrative staff, especially the Director of Intramural Research and the Associate Director for Program Management, have consistently taken the view that our travel restrictions are damaging to the program and wish to express to them my whole hearted support for their efforts, and at the same time urge them to continue to seek relief in every possible, legal way.

Dr. Cantoni in collaboration with Drs. P. Chiang, Y. S. Im, A. Guranowski, and G. Kunz have continued to explore various aspects of the biochemistry of S-adenosylhomocysteine, S-adenosylhomocysteine hydrolase with special attention to their role in the control of biological methylation.

S-Adenosylhomocysteine (AdoHcy) was first identified as one of the products of the reactions that utilize S-adenosylmethionine (AdoMet) as a methyl donor by Scarano and Cantoni in 1954. As is well known, AdoMet is one of the most versatile compounds in biology; it can function not only as a methyl donor in a very large number of methyltransfer reactions but also as a donor of a butyl amino side chain, of the amino group of adenosine, and, after decarboxylation, of a propylamine side chain. In addition, it is cleaved enzymatically to generate thiomethyladenosine and homoserine lactone and it can function in a variety of reactions as an allosteric effector. AdoHcy can inhibit competitively the great majority of these reactions and thus it must be considered as a key compound in the control of the cellular utilization of AdoMet.

In eukaryotes, AdoHcy is metabolized through a single metabolic pathway catalyzed by S-adenosylhomocysteine hydrolase, an enzyme that was first characterized by De la Haba and Cantoni in 1957, and recently crystallized from beef liver by Richards, Chiang and Cantoni.

The reaction catalyzed by S-adenosylhomocysteine hydrolase is reversible an the equilibrium is strongly in the direction of synthesis. Physiologically the reaction proceeds in the hydrolytic direction because the products of the reaction, adenosine (Ado) and homocysteine (Hcy), can be efficiently removed by a number of different enzymatic systems.

In vertebrates, and perhaps in insects, the hydrolysis of AdoHcy provides the only source of homocysteine that can be utilized in lieu of methionine as an essential amino acid and of cysteine, which is not an essential amino acid. Quantitatively, the formation of homocysteine and its hydrolysis are of maj significance since the flow of methionine through this pathway is of the orde of 300 μmoles/kg body weight/day.

With regard to the other product of S-adenosylhomocysteine hydrolase, adenos the interest in this compound has been greatly stimulated by the discovery in 1972, that an inborne error of metabolism, adenosine deaminase deficiency, is associated with severe immuno-deficiency involving both B and T lymphocytes.

The competitive inhibition of AdoMet transferases by AdoHcy implies that the intracellular ratio of AdoMet/AdoHcy must be important in the regulation of biological methylation. It has been calculated by Chiang and Cantoni that th consequences of a change in the AdoMet/AdoHcy ratio is very different for th different enzymes and depends both on the K_m for AdoMet and the K_i's for Ado Generally speaking, enzymes with a high affinity for AdoMet, and therefore with a K_m that is considerably lower than the intracellular concentrations o AdoMet, are more tolerant to changes in the AdoMet/AdoHcy ratio than enzyme that operate optimally at concentrations of AdoMet higher than its intracell concentrations.

Chiang, Richards and Cantoni have screened a large number of compounds for their ability to inhibit homocysteine hydrolase and identified among them a compound, 3-deazaadenosine (DZA), that is a very potent inhibitor of the enzyme. The inhibition is due to the fact that 3-DZA, like adenosine binds very tightly to the enzyme, but unlike adenosine it is not a substrate for adenosine deaminase or adenosine kinase, the two reactions, as indicated abo are principally responsible for the metabolism of adenosine.

As far as is known, all the effects of the administration of 3-DZA to a vari of cellular systems or to laboratory animals *must* be the result of perturbat of biological methylations. This interpretation is significantly strengthen by the fact that the biological and biochemical consequence of the administr tion of 3-DZA to cellular systems or laboratory animals are magnified by th simultaneous administration of homocysteine thiolactone. This compound ser intracellularly as a source of L-homocysteine and leads to the larger accumu tion of 3-DZA-Hcy. 3-DZA can, therefore, be used as a probe of the particip tion of biological methylation in a given biological process.

P. Chiang *et al.* have shown that a number of methylation reactions in rat li are inhibited after administration of 3-DZA. Specifically, they have found that administration of 3-DZA to rats will result in a drastic inhibition in methylation of phosphatidylethanolamine, without a decrease in the total ph phatidylcholine. Thus, observations lead to the conclusion that in the pre of an inhibitor of phosphatidylethanolamine methylation, the utilization of exogenous choline is markedly stimulated and reveals a hitherto unknown control mechanism that will require considerable further study and promises to be very interesting.

In addition to a variety of biochemical effects, 3-DZA exerts many different biological effects in a number of cellular systems through biochemical mechanisms that are as yet undefined, but that, as mentioned earlier, must involve perturbation of biological methylations.

Leonard and Skeel, in collaboration with Chiang and Cantoni, showed that 3-DZA at 1 μM level inhibits phagocytosis by resident peritoneal macrophages, and Chiang, Venkatasubramanian, Richards, Cantoni and Schiffmann have shown that chemotaxis in rabbit neutrophils in response to a chemoattractant tripeptide is inhibited under conditions where the binding of the tripeptide to the cellular receptors is not affected. Finally, Thompson, Chiang, Ruffolo, Cantoni and Nirenberg have shown that 3-DZA, and other inhibitors of AdoHcy hydrolase inhibit rapidly and reversibly synapse formation between retinal neurons in culture and myofibers. The inhibitory effect caused by 3-DZA was potentiated more than six times by addition of homocysteine thiolactone, a compound that when administered by itself had no effect.

In addition, it has been shown in a variety of tissue culture systems that 3-DZA exerts a powerful antiviral action against broad range of RNA and DNA animal viruses. With J. Bader, we have studied in some detail the antiviral activity of 3-DZA on RSV infection in chick embryo fibroblasts.

It is generally believed that the presence of a methyl group at the seven position of the terminal guanosine residue is important and in some cases essential for efficient translation of mRNA. It can, therefore, be suggested as a general working hypothesis that the efficacy of 3-DZA and other adenosylhomocysteinase inhibitors and/or adenosylhomocysteine analogs as antiviral agents relates to their ability to modulate or inhibit the reactions that lead to the synthesis of a fully methylated 5'-cap structure in viral mRNAs. Considerable experimental evidence supporting this hypothesis has been accumulated, but the critical demonstration of its validity is not yet available.

Drs. Mudd, Giovanelli and Datko, in the Section on Alkaloid Biosynthesis, as was the case last year, have continued to concentrate their research efforts upon achieving a better understanding of the biosynthesis of the "aspartate family" of amino acids, using *Lemna perpusilla* as the test organism. Aspartate is the starting point for the formation of lysine, threonine, methionine, and isoleucine. The first three are the three amino acids which are the "first-limiting" constituents in almost all protein-poor diets. Clearly, an understanding of this pathway and of the details of its regulatory relationships would be of importance in any rational approach to provision of better nutrition worldwide, since it is well established vertebrates are unable to synthesize methionine, an essential amino acid. Vertebrates, therefore, depend on a nutritional supply of methionine and derive this amino acid from plant sources.

Studies in this area during the past year have included: (a) Completion of establishment of optimal conditions for growth of *Lemna* and for carrying out labelling experiments of this organism with a fixed source of radioactive carbon. (b) Clarification of the life cycle of the individual *Lemna* colony under these standardized conditions, as well as definition of the structure of the population with respect to age and type of colonies in a growing sample of such colonies. A mathematical model has been developed which permits prediction of the population structure from knowledge of the individual life cycle. It has also been demonstrated that the net rate of protein synthesis does not vary greatly during the major phases of the life cycle of a *Lemna* colony, a finding which simplifies design of experiments with this plant. (c) Establishment of molybdate and selenate ions as reagents potentially useful in maintaining *Lemna* in steady-state growth with limited sulfate and/or in steady-state growth while producing selenium compounds of interest because they have the potential to act as false feedback inhibitors, or otherwise to disrupt normal regulation of sulfur metabolism. (d) Development of methods for analysis and degradation of methionine, threonine, and related compounds after labelling to isotopic equilibrium with ^{14}C-sucrose as sole carbon source. Development of these methods has been laborious, but they will permit performance of experiments of much greater power for study of the regulation of the aspartate pathway then would be the case by use of any other methods.

In the area of metabolic disease in humans, the study which seeks to determine whether heterozygotes for cystathionase synthase deficiency are at increased risk for development of coronary artery disease has been brought to the verge of completion. Within the next several months expansion of the two major control groups and collection of missing pieces of data should be completed. The information should then provide a realistic assessment of whether a moderate degree of homocystinemia does indeed lead to a substantial increase in risk for coronary artery disease. This assessment should be especially timely in view of current suggestions in the semipopular press that major changes in the American diet may be indicated to avoid such risk.

Klee and Streaty have developed a cell free preparation which acquires the biochemical attributes of opioid tolerance and dependence. Membranes, prepared from neuroblastoma x glioma hybrid NG108-15 cells, contain functional opioid receptors which remain coupled, as inhibitors, to adenylate cyclase. Incubation of these membranes with opioids for 30-60 minutes in the presence of Mg·ATP and a soluble protein fraction leads to the compensatory increase in adenylate cyclase activity. The membranes thus become tolerant to the inhibitory action of opioids and exhibit dependence as well since a naloxone challenge allows the expression of a supernormal amount of enzyme activity. This process appears to reproduce all of the essential features of opioid actions in the living cell. Because of the ease of manipulation of the cell free systems, it becomes possible to examine features of the mechanism of acquisition of opioid tolerance and dependence in molecular detail. Induction of dependence in this cell free system requires that more than half of the opioid receptors be occupied with opioid agonists. Thus, mixed agonists such as cyclazocine, nalorphine and buprenorphine do not lead to dependence in this system, in agreement with results obtained with animals. It has been found that the degree of dependence induction in the membranes is closely correlated to the agonist intrinsic activity of a large series of opioids. Thus this system may provide a simple and very rapid test for physical dependence liability of opioids as well as allow insights into the biochemical mechanisms involved. The direct quantitation of dependence liability has not, heretofore, been possible.

Knight and Klee have characterized two brain enzymes which can produce the opioid peptide enkephalin from larger precursors. Based upon regional distribution and kinetic considerations, one of these enzymes, associated with membrane components of the cell, may play a physiological role in the synthesis of this important neuropeptide. In the course of this work, a novel and generally useful assay for specific peptidase activities has been developed. The assay is based upon the availability of amphiphilic substrates so designed that they will adsorb to charcoal whereas one of the fragments of the desired cleavage is not. The amount of unadsorbed fragment is then measured by means of its radioactivity or spectral properties.

Zioudrou and Klee have continued their studies of opioid peptides derived from food proteins, the exorphins. Exorphins from wheat gluten and from casein have been extensively purified and efforts are currently underway to determine their structures. The opioid potency of gluten exorphin is very high in a number of assay systems and is quite comparable to that of the most potent known endogenous opioid peptides. Casein exorphin is much less potent, but is found in much higher amounts in pepsin digests than is the gluten peptide. The gluten stimulatory material which was discovered in the course of these studies has now been unequivocally identified as adenosine, a substance known to have powerful pharmacological and physiological actions which often antagonize those of the opiates. In a related study, carried out in collaboration with Rapoport and his colleagues, the permeability of the blood-brain barrier to opioid peptides has been measured and found to be high enough to allow equilibration in 15 minutes or so. Thus opioid peptides can reach the central nervous system from peripheral sources. The extent to which they normally must still be determined.

Dr. Merril continued to study induction of the galactose operon, under the control of lambda bacterial virus. In collaboration with Drs. M. Gottesman and S. Adhya, the locations of the three enzymes of the Leloir galactose pathway have been found in two-dimensional electrophoretic patterns from crude cell lysates. The positions of these enzymes was determined by electrophoresis of a collection of isogenic strains. Some strains had amber mutations in the structural genes for the enzymes of the Leloir pathway. The two-dimensional patterns were analyzed for the location of the missing enzyme spot, when an amber mutation was present in the enzyme structural gene. The position of the kinase enzyme has been corroborated in with electrophoresis of a double antibody precipitate, with antibody made to purify *E. coli* gal-kinase.

Clinical applications of the two-dimensional electrophoresis technique, in collaboration with Drs. Ebert and Polinsky, were made difficult because of the inability of existing protein stains to detect trace proteins. Florescent stains were tried with little, if any, increase in sensitivity over the commonly used stain, Coomassie Blue. However, by adapting a histological silver stain, with Drs. R. Switzer and S. Shifrin, a protein stain 100 times more sensitive, the Coomassie Blue was developed. This stain has greatly facilitated clinical investigations of genetic disorders.

ANNUAL REPORT OF THE LABORATORY OF NEUROBIOLOGY
National Institute of Mental Health
July 1, 1978 to September 30, 1979

Ichiji Tasaki, M.D., Ph.D., Chief

The Laboratory of Neurobiology has been studying the mechanism of nerve excitation and conduction from a macromolecular viewpoint. The research included both electrophysiological and biochemical research projects.

(A) The electrophysiological techniques used include: (i) Further study of the miniature responses (1-30 µV in amplitude) of nerve recorded by means of a realtime spectrum analyzer; (ii) the use of the perfused squid giant axon preparation to study the bi-ionic action potential; and (iii) the use of artificial colloidin membranes to study asymmetry currents.

(B) The biochemical techniques were used to study: (i) Phosphorylation of proteins from nerve membrane; (ii) calcium-activated protease found in the axoplasm of squid giant axons; (iii) binding of calcium to membrane fragments isolated from lobster nerve; and (iv) binding of saxitoxin to membrane fragments isolated from lobster nerve.

A. Electrophysiological Studies

(i) The mechanism of generation of periodic miniature responses was investigated extensively by the use of the technique of intracellular perfusion invented in this laboratory. To detect small electrical signals generated by squid axons, recent models of Fast-Fourier-Transform analyzers manufactured by Nicolet Scientific Corporation, New Jersey, were employed. With the present experimental setup, the Fourier components of the random noise produced by the recording electrodes, the preamplifier and the analyzer were between 0.5 and 1 microvolts in amplitude. The following reagents which chemically alter the primary structure of membrane protein molecules were used to generate miniature responses: N-bromosuccinimide (which cleaves tryptophanyl peptide bonds primarily), glutaraldehyde (which cross-links protein molecules), dye-mediated photo-oxidation (which attack histidine), etc. At present, a thermodynamic study is in progress which explains the mechanism of the elementary process of repetitive excitation in terms of conformational changes of the intramembrane protein molecules.

(ii) Under intracellular perfusion with a solution containing potassium as the sole cation species, squid giant axons were found to be capable of developing all-or-none action potentials when immersed in a medium in which $CaCl_2$ was the only electrolyte. The external Ca-ion could be replaced with cobalt or manganese ion without eliminating the ability of the axons to develop action potentials. The reversal potential measured by the voltage clamp method varied directly with the logarithm of the concentration of extracellular Ca-ion; the proportionality constant was close to 29 mV for a 10-fold change. It is inferred that the electrochemical process underlying the generation of these Ca-K bi-ionic action potentials is an abrupt change in the ratio of mobility of Ca-ion to that of K-ion in the membrane.

The excitability of squid giant axons perfused internally and externally with an identical solution which contained divalent cation salt of one kind only was demonstrated by passing a constant inward current through the axon membrane. Salts of cobalt, manganese, nickel and barium were suitable for maintaining the excitability. This simple ionic condition offers a great advantage in developing a physico-chemical theory of the nerve excitation.

(iii) The artificial membranes were used to study the asymmetrical currents generated when voltage pulses of equal amplitude but opposite polarity were applied across the membrane. When the salt species in the solutions bathing the membrane were diffent on either side of the membrane, large current asymmetries were found. These asymmetries could be explained by considering the difference in mobility of various ions. These results suggest an alternative interpretation for the recently reported asymmetric current in squid giant axons generally known as gating currents.

B. Biochemical Studies

(i) The major findings from the project involving phosphorylation of proteins from nerve were described in last year's report. This project has now been extended to include the phosphorylation of synaptosomaes isolated from squid brain.

(ii) The properties of a calcium-activated protease found in the axoplasm of squid giant axons were studied. The protease in the 100,000 x g supernatant of axoplasm is very specific and degrades only the 200,000 m.w. neurofilament protein (NF_{200}), whereas the protease(s) in the pellet has a much wider range of substrate specificity. The activation of the supernatant protease is restricted to Ca^{2+} ion, and no other divalent cation substitutes Ca^{2+}. The protease requires > 0.5 mM Ca^{2+} for activation and has a pH optimum of about 7.5. Degradation of the NF_{200} appears to proceed through a 100,000 m.w. and possibly a 47-50,000 m.w. intermediate form before degradation to trichloroacetic acid soluble peptides. Activity of the protease is inhibited by divalent cation chelators, Cu^{2+} and Fe^{2+}, sulphydryl inhibitors and leupeptin. This specific Ca^{2+}-activated protease in squid axoplasm has properties identical to a Ca^{2+}-activated protease found in various non-neural tissues. Despite its high protein substrate specificity, the Ca^{2+}-activated protease purified from human platelets effectively degrades squid NF_{200}, suggesting a possible relationship between platelet and muscle actin-binding proteins and neurofilament proteins.

(iii) The binding of calcium-45 to membrane material isolated from lobster walking leg nerves was studied using a rapid filtration technique. In solutions of high ionic strength (450 mM), the amount of calcium-45 bound to this membrane material was found to be highly dependent on the monovalent cation used in the incubating solution. The amount of calcium-45 bound was larger when the membranes were incubated in a KCl solution compared to when they were incubated in a NaCl solution. This difference was attributed to the ability of these closed membrane vesicles to accumulate calcium into the vesicle when incubating in a KCl solution but not in a NaCl solution. This accumulation of calcium was found to be independent of metabolic energy. At low ionic strength, the membranes formed open fragments and the amount of calcium bound was no longer sensitive to the monovalent cation species in the incubation solution. The calcium-45 bound under these low ionic strength conditions was considered to be bound to anionic sites on the membranes.

(iv) The findings from the research project involving the binding of saxitoxin to nerve membrane are as follows. Saxitoxin is a potent neurotoxin which binds to sites on the membrane involved in excitability. It is thus a very convenient biochemical probe for the study of these sites. The mechanism by which phospholipase A$_2$ inhibits the binding of ^{3}H-STX to membranes from lobster walking leg nerves was examined. It was concluded that this inhibition of the binding was due to the accumulation of unsaturated fatty acids and lysophosphatides, products of the lipase action, into the ^{3}H-STX binding site. The destruction of the lipids themselves did not appear to cause the loss of the binding. The following evidence was presented in support of these conclusions. 1) Among the phospholipases tested, phospholipase A$_2$ was the most effective in inhibiting the binding. Phospholipase A$_2$ is also the only lipase which produces fatty acids and lysophosphatides. 2) The addition of fatty-acid-free bovine serum albumine (BSA) protected the ^{3}H-stx binding sites from the inhibitory action of phospholipase A$_2$: fatty-acid-free BSA is capable of binding free fatty acids and lysophosphatides, thus preventing these products from interacting with the STX binding sites. 3) The addition of exogenous unsaturated fatty acids or lysophosphatides to membrane material inhibited the binding. These findings show that the STX binding activity is sensitive to unsaturated fatty acids and to lysophosphatides.

Annual Report of the Laboratory of Neurochemistry
National Institute of Mental Health
October 1, 1978 - September 30, 1979

Seymour Kaufman, Chief

During the last year one of the major efforts of the Laboratory of Neurochemistry has been the continuing study of both the clinical and basic aspects of the mammalian phenylalanine hydroxylase system.

We have in the past developed assays that permit the rapid detection of hyperphenylalaninemia (HPA) due to biopterin deficiency. The test is based on the observation that normals, as well as patients with classical PKU and those with HPA due to dihydropteridine reductase (DHPR) deficiency, show an increase in blood levels of biopterin when their blood phenylalanine levels increase. In contrast, patients with HPA due to biopterin deficiency do not show this response.

Although this test represents an important advance over previous diagnostic methods, which are based on cofactor assays on liver biopsy samples, the procedure does involve administration to infants of large doses of phenylalanine and the withdrawal of multiple blood samples. For diagnosis of HPA due to DHPR deficiency, our previously developed enzyme assay on cultured fibroblasts, a procedure that takes 7 or 8 weeks, has been the only alternative to liver biopsy.

Recently we have succeeded in developing new assays for the differential diagnosis of HPA due to DHPR deficiency and HPA due to biopterin deficiency. The test for the latter condition is based on determination of the neopterin to biopterin ratio in urine, a high ratio being characteristic of the disease. The test for the former condition is based on the failure to detect tetrahydrobiopterin in a fresh urine sample from patients with the disease. Both tests can be carried out on small samples of urine, do not involve administration to the patients of any substance, and provide results within a few hours. They are, therefore, quick, accurate and non-invasive and are already being used clinically.

The results on which the new procedure for detection of HPA due to biopterin deficiency are based, namely the observation that these patients accumulate large amounts of neopterin in their urine, provide another example of the dynamic interrelation between basic and clinical research. Our finding that neopterin accumulates in these patients indicates that they are blocked in the biopterin biosynthetic pathway between neopterin and biopterin and probably constitutes the strongest evidence to date that neopterin is the normal precursor of biopterin.

We have extended our earlier studies on the *in vivo* regulation of phenylalanine hydroxylase by phosphorylation-dephosphorylation mechanisms by showing that an approved drug, theophylline, when given to rats elicits a prompt increase in the activity of hepatic phenylalanine hydroxylase. The catalytic and physical properties of the enzyme from the drug-treated rats indicate that the activated enzyme is more highly phosphorylated than the control enzyme. These results indicate that it may be possible to use this drug to increase the activity of phenylalanine hydroxylase in atypical PKU patients who have residual hydroxylase activity.

Dr. Neville and his colleagues have continued to study the possibilit of using receptor-mediated protein transport as a way of developing a new type of specific pharmacologic agent. The underlying premise of this approach is that a receptor on the cell surface which mediates the entry into the cell of a specific protein can also mediate the entry of other proteins that are linked to that specific protein, and that after it has entered the cell, the linked protein remains functional. They have valida this premise by showing that a hybrid molecule composed of ricin and diphth toxin fragment A can enter cells via the ricin receptor and that the toxin can express its biological activity within the cell. These studies show that it may be possible to prepare new cell-specific pharmacologic agents with the use of hybrid protein technology.

Dr. Nash and his coworkers have continued to make progress in the analysis of the system that carries out integration of the DNA of a virus into the chromosome of its bacterial host. They have made the important finding that Int protein, the product of the bacterial virus int gene, is a topoisomerase, i.e., this protein acting by itself can break and reseal the strands of DNA. Furthermore, the topoisomerase activity of Int is very similar to that of enzymes that have been detected in the nuclei of animal cells. This finding suggests that these enzymes, whose roles until now have not been elucidated, may be involved in genetic reco bination in eukaryotes.

Annual Report of the Laboratory of Neurophysiology
National Institute of Mental Health
Edward V. Evarts, M.D., Chief
October 1, 1978 -- September 30, 1979

The projects of the Laboratory of Neurophysiology may be categorized according to two major areas of ongoing research: I) Brain Mechanisms in Movement and II) Functional Neuroanatomy. The work on movement is aimed at providing an understanding of the brain's motor control system, a system consisting of three major components (cerebral cortex, basal ganglia, and cerebellum). Research currently in progress in LNP is providing knowledge as to how the three components function in a coordinated way during movement. Such knowledge, essential to rational search for improved treatments of a number of psychomotor disorders, is being acquired by means of investigations of brain physiology and brain anatomy. Our studies of brain physiology involve microelectrode recordings of nerve cell discharge in monkeys performing learned movements. These studies have shown that cells in each of the three major components become active prior to voluntary movement. The entire cerebral cortex has massive nerve fiber connections to both the basal ganglia and the cerebellum, and these two structures in turn have powerful connections back to the motor cortex by way of the thalamus. Thus, the basal ganglia and the cerebellum receive information from the somatosensory, visual, and auditory regions of the cerebral cortex, transform this information, and then send a new pattern of signals to the motor cortex. Whereas the traditional view held that the cerebral motor cortex was at the highest level of motor integration and that the subcortical structures were at a lower level, that is, functionally closer to the spinal cord and muscle, it now appears that the situation is quite the reverse.

In addition to our studies of brain cell activity during voluntary movement in monkeys, we are devoting a major effort to collaborative research with clinicians seeking to obtain a better understanding of a variety of human illnesses in which disorders of movement constitute an important problem. Our work deals with illnesses such as tardive dyskinesia, parkinsonism, Tourette's syndrome, Huntington's disease, and other basal ganglia diseases. The goals of the work are: (1) to gain further understanding of the brain disturbances underlying the disorders, (2) to improve diagnostic and prognostic procedures in relation to motor disorders, and (3) to establish objective measures for assessing effectiveness of treatment.

In conclusion then, our work on movement involves basic research in monkeys to acquire knowledge concerning the relationships between brain areas controlling movement: experiments in monkeys allow the brain to be probed using microelectrodes and neuroanatomical techniques. In contrast, studies of the pathophysiology of movement in patients are carried out using noninvasive techniques which emphasize observations on the way in which therapeutic agents (e.g., L-dopa in Parkinson's disease) modify the underlying pathophysiology.

A second and expanding area of research in LNP is Functional Neuroanatomy. This work has involved a) tracing of neuronal fiber connections in the brain, b) development of a new *in vitro* autoradiography technique for localizing opiate and other peptide receptors in the brain, and c) localization of brain sites which are selectively involved in sleep and drug-induced sedation. The fiber-tracing technique, based on transport of tritiated amino acids, has be used to show by autoradiography a variety of laminar distributions of thalamic projections to cerebral cortex. These data provide structural evidence for mechanisms of thalamocortical integration. In addition, a new method for in vitro localization of brain receptors within slices of fresh-cut brain tissue is being developed. The technique has the potential for providing a simple and general way of visualizing sites of action of a wide range of biologically active substances in any fresh brain tissue. Receptor sites for opiate derivatives and benzodiazapines are currently under investigation. A third application of the autoradiographic technique will be to visualize brain areas with special functional and metabolic characteristics. Labeled 2-deoxy glucose may reveal brain sites that have special metabolic properties during sleep states following various forms of experiences by rats.

LNP work on Functional Neuroanatomy also involves a broad, coordinated neurophysiological and neuroanatomical examination of the corticocortical connections of the sensorimotor cortex and their role in primate motor behaviors which rely upon sensory input. This work seeks to answer four major questions: first, the influence of the densest of the corticocortical inputs to the primary motor cortex (MI) is being examined by reversible cooling of the primary somatic sensory cortex (SI) and posterior parietal cortex (area 5), while recording the response of single neurons in the MI cortex to somatic sensory stimulation. Second, the responsiveness of neurons in the primary motor corte and the supplementary motor cortex (MII), which both receive dense corticocortical inputs from somatic sensory areas of the cortex, is being directly compared in the alert, behaving monkey. Third, the precise responsiveness of neurons in area 3a, the "transition" cortex between MI (precentral cortex) an SI (postcentral cortex), to somatic sensory stimulation and the extent of its corticocortical projection to MI is being investigated. Fourth, the efferent connections of MII, especially its corticocortical and corticothalamic projections, are being examined in an effort to determine the extent to which these connections differ from those of MI.

The individual projects in progress within each of these broad areas are as follows:

I. Brain Mechanisms in Movement

1. The role of motor cortex in control of voluntary movement
2. Eye-head coordination in the primate: neural substrates and plasticity
3. Role of somatic sensory inputs in the cerebral control of movements
4. Motor function in patients with neuropsychiatric disorders

II. Functional Neuroanatomy

1. Corticocortical connections in the sensorimotor cortex of the monkey
2. Studies of central nervous system functional anatomy

In the summary which follows, the significance of each of these projects will be considered.

1. The Role of Motor Cortex in Control of Voluntary Movement

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in basal ganglia and cerebellum.

Of the two major classes of inputs impinging upon the cerebral motor cortex and generating the stream of impulses passing to the spinal cord, that class of inputs which operates automatically and constitutes the afferent limb of the transcortical servo loop seems the simplest to understand. This transcortical servo operates according to Sherringtonian principles of reflex action. The second set of inputs passing to motor cortex via the thalamus depends upon basal ganglia and cerebellum, and is much more complicated. In order to understand voluntary movement we need to comprehend the sorts of information processed by cerebellum and basal ganglia, and to discover the way in which the outputs of cerebellum and basal ganglia interact in the thalamus. It is these problems which stand at the forefront of studies on brain mechanisms of movement.

2. Eye-Head Coordination in the Primate: Neural Substrate and Plasticity

The long range aim of this project is to determine the neural basis of adaptive mechanisms responsible for maintaining appropriate performance levels in the oculomotor system. One project is concerned with localizing the modifiable elements subserving long-term regulation of the gain of the vestibulo-ocular reflex. Single unit recordings in the vestibulo-ocular pathways of awake monkeys have revealed that the underlying modifications must occur beyond the first synapse in the medial vestibular nuclei. The second project is concerned with the mechanisms by which the static (step) component of saccadic eye movements is appropriately matched to the dynamic (pulse) component to insure stable vision following such eye movements. By recording eye movments and shifting the visual scene after each saccade, it has been shown that persistent post-saccadic retinal image slip is sufficient to induce pulse/step mismatch, suggesting an important role for visual inputs in this adaptive process.

A great deal is known about the anatomy and physiology of eye movements. The discrete, rather machine-like quality of the oculomotor system has proved particularly amenable to the analyses of information processing at the single neuron level. The fact that the system also displays plasticity opens up new approaches to the subject of motor learning at the cellular level in the primate central nervous system. The single unit recording studies are being undertaken to establish the exact locus of the modifiable elements in the vestibulo-ocular reflex, a necessary first step before it will be possible to investigate the underlying cellular mechanisms. The studies on the saccadic system are at an earlier stage of development and have established the potential significance of visual inputs in the adaptive regulation of these eye movements. The latter may prove to be of clinical significance since the kind of disruptions which we have induced by purely visual means are seen in some pathological cases (for example, VIth nerve palsies of recent origin) and in some strabismic patients recovering from corrective surgery.

3. Role of Somatic Sensory Inputs in the Cerebral Control of Movements

This project is a study of the role of sensory inputs in the cerebral control of motor behavior in the primate. It uses the technique of single neuron recording in behaving monkeys to directly compare the activity of neurons in primary motor cortex (MI) during the performance of learned arm movements with the activity of neurons in the parts of the somatic sensory (SI), parietal and frontal cortex which are reciprocally connected with MI. Two questions form the focus of our investigations. First, the possible modulation of peripheral inputs to the sensorimotor cortex during motor activity is being studied by recording SI and MI response to peripheral stimuli delivered during and before active movements, during different levels of steady state motor activity related to differences in the exerted force or length of the contracting muscles and during isometric versus isotonic contractions in which the animal controls either exerted force or arm position. Second, the relation of posterior parietal and frontal cortical areas to visually guided movements is being investigated by determining whether the neuronal activity in these areas reflect information concerning the visual cue to begin movement, as well as limb position or exerted force.

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in motor control, more information is needed concerning the characteristics of sensory input to MI, the pathway taken by this input and its role in the initiation and control of motor behavior. As an important step towards this goal, the present project examines several cortical areas through which peripheral inputs could influence motor cortex output.

4. Motor Function in Patients with Neuropsychiatric Disorders

The relevance of movement disorders to neuropsychiatric illness has become increasingly apparent with the discovery that symptoms of Parkinson's disease may occur as side effects during therapeutic administration of neuroleptic drugs and that cessation of neuroleptics may be followed by tardive dyskinesia, The potential value of information concerning such neurochemically determined motor disorders is twofold: 1) at an applied level, quantitative measures of motor function will be useful in drug dosage regulation of therapeutic agents and in drug effectiveness comparisons for different agents; 2) at a basic level, discovery of mechanisms of motor disorders will help us to understand the brain disturbances underlying the wide range of neuropsychiatric illnesses related to neurochemical disturbances. Studies thus far have focused on obtaining quantitative indices of the fluctuations in effectiveness of antiparkinsonian drug therapy. Tests of movement speed and reflex responses to limb displacement have provided us with measures which correlated with clinically determined fluctuations in drug efficacy, pointing to the feasibility of setting up routine tests of motor function to be administered in clinical settings.

The methods for motor function evaluation which we have developed will be applicable to large samples of patients and normal control subjects, and will provide data which can be stored in computerized form. As such, the measures to be obtained will be of potential value for studies such as the study currently being undertaken by the NIMH in New Haven. This study, in collaboration with the NIA and NINCDS, is currently underway to select a group of elderly subjects who exhibit psychological and/or neurological disturbances associated with aging. At the present time this study is in its early stages, with initial subject selection to be carried out on the basis of interviews of thousands of persons. In the next phase of the study, neurological examinations will be carried out on those subjects who on the basis of interview appear to have clinical disturbances. It is at this second stage of the study that the NINCDS will enter the picture.

In a third stage of the project, subjects who on the basis of neurological examination fall into categories such as Alzheimer's disease, presenile psychosis, or early Parkinson's disease, will be studied further. It is at this stage that our systems for motor function evaluation would come into play. The systems would provide objective uniform tests of motor function which would make it possible to compare a number of different clinical categories.

5. Corticocortical Connections in the Sensorimotor Cortex of the Monkey

The somatic sensory inputs to the primary motor cortex (MI) are thought to play a significant role (perhaps by a negative feedback mechanism) in the regulation of precise movements in the monkey. The source of this input is not known and it is the objective of the present project to obtain some information pertinent to this question. Furthermore, there is a more general question concerning the role of sensory inputs to the entire motor cortex (including cortical motor areas other than the primary motor cortex) of sensory modalities other than the somatic sensory. It has long been known that in addition to the MI motor representation, there exists at least one other motor representation (MII, the supplementary motor cortex); and recent evidence suggests the existence of as many as four additional representations. These motor areas may be influenced by somatic sensory or visual inputs, since it is known that each of these areas receives monosynaptic corticocortical inputs from either the somatic sensory cortex or the parts of the posterior parietal cortex related to somatic sensorimotor or visuomotor activities. The present project was formulated in an attempt to determine the significance o sensory corticocortical inputs to various parts of the motor cortex and thei role in the production and regulation of the precisely controlled movements characteristic of primates.

About 500 units have been recorded in two monkeys tested for this project and five findings have emerged: (1) The somatic sensory or posterior parietal cortex (areas 2 and 5) and its projection to MI is necessary for the somatic sensory responsiveness of certain neurons in the MI cortex. Thus, this corticocortical projection may play a substantial role in any feedback mechanism whereby motor behavior is modified or regulated by somatic sensory inpu (2) Many neurons in MI are not significantly influenced by cooling inactivation of area 2 and 5 and therefore probably receive their somatic sensory input via the transition cortex between MI and SI (i.e., area 3a) or some other thalamic or cortical source. (3) Units in area 3a may be identified by their approximately directly proportional response to a graded series of imposed velocities of foot movement. Such a method of identification of area 3a will allow a controlled neuroanatomical investigation of the cortical and thalamic connections of this cortical area. (4) Though the MII and MI cortex receive a monosynaptic input from almost every known somatic sensory representation in the cortex, neurons in MII are much less responsive than those in MI to peripheral somatic sensory stimuli of the type delivered in this study. Since the peripheral stimulus which we have applied is complex and encompasses many somatic sensory submodalities, it is likely that our finding is a general one. This observation raises significant questions concerning the role of the corticocortical connections between the somatic sensory cortex and MII. (5) Certain observations have led to a modification of the currently accepted view of th topographic organization of the motor "maps" in the parts of the MI and MII cortex concerned with the hindlimbs and tail, and have clarified the relationship of these physiologically defined cortical fields and the cortical fields defined anatomically (cytoarchitecturally), a relationship which has been obscured in the recent past.

6. Studies of Central Nervous System Functional Anatomy

One phase of this project uses histochemical tracing techniques to map neuronal connections within the brain. Work in progress has dealt with the cortical projections of thalamic nuclei, which collectively may be called the "nonspecific" thalamus. The cell groups under investigation--the midline, intralaminar, parafascicular, ventromedial and ventrolateral nuclei--attracted interest over 30 years ago when electrophysiologists found that electrical stimulation of nonspecific thalamic sites in the cat could elicit widespread and synchronous cortical responses. The data further suggested that the pathways carrying these electrical events to the cortex were not only widely distributed, but were anatomically separate from the known thalamocortical pathway originating in the "specific" thalamic relay nuclei. Moreover, microelectrode depth recordings suggested that specific and nonspecific afferent fibers terminate in separate cortical layers. Unfortunately, however, the neuroanatomical tract tracing techniques, then based on degenerating axons, could find little evidence for nonspecific thalamocoratical projections terminating in wide areas and in unique laminar patterns. Consequently, the early workers abandoned their interest in the possibility that these mechanisms could in part underli cortical and behavioral arousal phenomena, and even conscious integration. The present studies have provided the long-sought anatomical bases for the electrophysiological phenomena, for it has been found that there is a remarkable and unique projection to the most superficial layer of almost the entire neocortex originating from the ventromedial thalamic nucleus in the rat. Another widespread and hitherto unrecognized projection to the hippocampus and limbic cortices was found after placing a mixture of ^{3}H-leucine and ^{3}-proline into the nucleus reuniens of the thalamic midline group. Results from autoradiographic cases of isotope injection into intralaminar thalamic nuclei suggest another instance of widespread neocortical distribution but, in contrast to that of the ventromedial nucleus, intralaminar projections terminate in deep cortical layers. Some results suggest that thalamic inputs may be distributed to two or even three layers, and current experiments are aimed at determining whether the multiple and spaced laminations arise from a single or adjacent thalamic cell groups.

A second phase of work in functional neuroanatomy has involved an extensive collaboration with Dr. Candace Pert and her coworkers in the Biological Psychiatry Branch of the NIMH. Efforts have been aimed at developing new methods to localize specific brain regions, the sites of action of pharmacologically active drugs, and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of anatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and the fiber pathways and terminal distributions in any given region. This search for anatomical correlates is augmented by the use of immunocytochemical mapping techniques for selectively marking cells and axons containing the putative endogenous transmitters that are released onto the receptors. The assignment of a neurotransmitter to each of the identified CNS pathways is the ultimate goal of this type of effort.

The radioassay of dissected cortical layers has revealed the laminar distribution of receptors for bombesin, vasoactive intestinal peptide (VIP), diazepam (Valium) and β-noradrenalin (clonidine). The most exciting result is the high concentration of VIP receptors in layers I and VI of the cortex, since these are areas that receive the nonspecific thalamocortical projections. Thalamic lesions have been made unilaterally to assess the receptor content after deafferentation of the thalamic inputs, but so far the results have been equivocal: one study showed apparent receptor supersensitivity on the lesion side, but subsequent replication attempts failed to do so.

The study of the brain localization of radioactively labeled substances such as 2-deoxy-D-glucose, peptides and diazepam, is an emerging hybrid of recently developed histochemical and biochemical techniques. In each instance the knowledge to be gained permits new insights into functional neuroanatomy. Previous efforts to elucidate structure-function relationships were limited by the paucity of useful techniques. Autoradiographic localization of biologically and pharmacologically active substances provides a more powerful tool for observing how the central nervous system operates.

Annual Report

Laboratory of Clinical Psychopharmacology

Richard J. Wyatt, M.D., Chief

The year 1979 has been an extremely productive one for the Laboratory. Drs. Gillin and Mendelson finished their work on the Institute of Medicine Report on Barbituates, which received praise from many sources. Dr. Gillin, together with Dr. Sitaram, in the Bethesda Sleep Unit, continued their excellent work on acetylcholine, memory and REM sleep (the report of this can be found in the Annual Report of the Laboratory of Biological Psychiatry). Dr. Sitaram won the A.E. Bennett award for the best clinical research done by a psychiatrist under 35 years of age--the second straight year a Clinical Associate connected with our program has received this prestigious honor.

Dr. Reynolds and his staff spent a good portion of the year getting ready for the St. Elizabeth's Hospital Accreditation Review. The review took place in early June and the WAW Division came off extremely well. Dr. Weinberger's finding of several different CT scan abnormalities in chronic schizophrenics is a reawakening of interest in brain structure and schizophrenia. This was an area of major research 40 years ago but interest in anatomy decreased with the pharmacologically stimulated work on the synapse in the late 1950's. Similarly Dr. Luchins used the CT scan to demonstrate left-right brain asymmetries in milder forms of schizophrenics.

Dr. Kleinman found an increased correlation between plasma prolactin and platelet MAO activity. This indicates that platelet MAO reflects a functional brain alteration.

Drs. Potkin and Karoum found that an endogenous amphetamine-like agent, PEA, was increased in the urine of some chronic schizophrenic patients. Paranoid schizophrenic patients had higher concentrations of PEA than non-paranoid patients.

Drs. Wise and Potkin developed a new more reliable platelet MAO assay than was previously available to us. Drs. Neckers and DeLisi developed a lymphocyte MAO assay which shows a 10 fold alteration in activity between schizophrenics and controls compared to the 50 percent decrease found with the platelet.

Drs. Perlow and Freed in collaboration with Drs. Hoffer at the University of Colorado and Drs. Sager and Olsen at the Karolinska Institute successfully grafted brain tissue from one animal to another changing the behavior of the host rat. There was no rejection of the grafted tissue.

Finally a very good piece of news comes as I am writing this report. A small number of positions have become available for beginning our longplanned research in geriatric pshchiatry. These resources will give us an opportunity to make an advance in a very important field.

LABORATORY OF PRECLINICAL PHARMACOLOGY

CHIEF: Erminio Costa, M.D.

1978 -- 1979

Novel lines of investigation initiated during the previous fiscal year, have reached fruition during 1979 and have contributed to a better understanding of a number of mechanisms that are operative in neuron to neuron signaling. In addition, these experiments have unveiled that an increased synthesis of the enkephalin-like peptides stored in N. accumbens and corpus striatum participates in the long term effects of neuroleptics. Indirectly, this finding supports the theory that a defect in the enkephalin production might be associated with certain psychotic states. Because of this possible interaction, we became interested in studying how enkephalins interact with postsynaptic receptors. For these studies, Dr. Guidotti and collaborators used as a model the adrenal medulla which is innervated by cholinergic and enkephalinergic neurons and contains chromaffin cells which contain acetylcholine (ACh) and opiate receptors in their membranes. Studies by Dr. K. Kumakura have revealed that enkephalin released onto chromaffin cell membranes may function as a disabler of cholinergic receptor function. Using primary cultures of chromaffin cells, Dr. Kumakura could show that opiate peptides including beta-endorphin, could inhibit the secretion of catecholamines elicited by nicotinic receptor stimulation. In this line of investigation, the discovery made by Dr.Di Giulio that the adrenal medullae or sympathetic ganglia and arteries of spontaneously hypertensive rats contain less enkephalin-like material, has suggested that enkephalin-like peptides may play a role in the regulation of cardiocirculatory function in health and disease states. These findings were made possible by studies of Dr. Yang and her colleagues showing that peripheral tissues of rats, guinea pigs, calves and rabbits can store polypeptides which cross react with [met^5]-enkephalin and [leu^5]-enkephalin antibodies. Work by Dr. Yang suggests that the adrenal medulla contains two main pools of enkephalin-like material; these pools have been characterized anatomically and biochemically. The pool of enkephalin-like material which is located in the terminals of the splanchnic nerves can be characterized chromatographically and immunologically as a rather homogeneous pool of [met^5]-enkephalin, in contrast, the other pool which includes mainly high molecular weight enkephalin-like material, evinces a high degree of molecular heterogeneity and it is located almost exclusively in the chromaffin cells. Enzymatic, immunological, chromatographic and chemical criteria indicate that a new peptide which cross reacts with [met^5]-enkephalin antibodies is present in chromaffin cells. This peptide was partially purified and was tested for its biological activity. It was found that it lowers the blood pressure of rats when injected intravenously in the deeply anesthetized animal (in collaboration with Drs. Hanbauer & Gaida, NHLBI), inhibits electrically-induced contractions of guinea pig intestine and induces wet dog shakes and hypotonic catatonia when injected intraventricularly. All these responses are naloxone resistant; therefore it is possible that the natural receptor for this peptide is different from the antinociceptic receptor. The peptide is being characterized further because we suspect that it might play an important role in the function of adrenal medulla. This view is supported by the finding that a similar polypeptide is present in blood plasma. Since enkephalin-like peptides stored in various tissues are decreased in spontaneously hypertensive rats, and since this change occurs early in life and certainly several weeks before the increase in blood pressure becomes evident (4 weeks of age), one can infer that the reduction in the enkephalin-like material is not a consequence of hypertension.

Another line of investigation which has reached fruition was carried out by Dr. Schwartz in collaboration with Dr. X. Breakfield (Yale University, New Haven, Connecticut) was directed to clarify the molecular defects operative in familial dysautonomia. Using cell lines of human fibroblasts derived from normal volunteers and patients, they found that the nerve growth factor (NGF) stored in these two types of cells is different. The NGF of cells from dysautonomic patients has a lower degree of biological activity per immunoreactive unit. Since NGF has trophic effects on sensory and catecholamine neurons, this finding could help enhance our understanding of the pathogenesis of sensory and sympathetic tone disturbances predominantly evident in the symptoms exhibited by patients with familial dysautonomia.

Several lines of independent evidence show that postsynaptic receptors change their affinity to specific neurotransmitters and thereby participate in the instantaneous modulation of the efficiency of synaptic transmission. During normal activity the affinity of the recognition site for the neurotransmitter oscillates within a spectrum of variable values; this spectrum delineates maximum and minimum limits of sensitivities which are termed sub- and supersensitivity. In addition to the change in affinity, there are a number of additional molecular mechanisms operative in determining sub- and supersensitivity of receptors. Dr. Chuang has found that in frog erythrocytes internalization of recognition sites and successive degradation play a major role in causing the subsensitivity of beta-adrenergic receptors elicited by protracted receptor stimulation with specific agonists. This finding suggests that in some receptors, subsensitivity can be due to an acceleration of the normal process for receptor degradation which is not associated with an increase in the synthesis rate of receptor recognition sites. In fact, we suspect that receptor internalization emphasizes a normal mechanism that is operative in receptor turnover. Drugs may act on receptor plasticity by changing the affinity and internalization rates of the recognition sites and also by modifying a number of other mechanisms. For instance, Dr. Toffano (guest worker from Italy) has shown that GABA receptors exist in two conformational states which correspond to a different affinity for the agonist. Benzodiazepines facilitate the conformation with a high affinity for the GABA receptors by competing with a protein that down regulates high affinity GABA receptors. In this down regulation, an important role is played by membrane protein phosphorylation which is efficiently inhibited by the protein which down regulates GABA receptors. Therefore, when this protein is present in the membrane, by inhibiting membrane protein phosphorylation, it suppresses the expression of high affinity recognition sites for GABA. Operationally, we have termed this protein GABA-modulin to emphasize its regulatory capacity on GABA receptors. The protein was purified by Dr. Leon (guest worker from Italy) -- she obtained an increase in the specific activity of GABA receptor modulation and phosphorylation by about 1500-fold. These results suggest that the balance between low and high affinity GABA receptors is determined by the degree of phosphorylation of some membrane protein that has not yet been identified. Purified synaptic junctions contain Ca^{2+} dependent protein kinase activity which is greatly increased when the GABA-modulin content of the synaptic junctions is reduced by repeated washings. That protein phosphorylation is relevant in GABA receptor modulation was also shown by preventing the down regulation of the GABA binding elicited by GABA-modulin by previously phosphorylating the synaptic junctions. How phosphorylation of membrane protein participates in the regulation of the recognition sites of GABA receptors is presently being studied in a neuroblastoma cell line. This clone contains a GABA receptor unit composed of GABA recognition sites, benzodiazepine recognition sites, protein kinase, Cl^- ionophore and GABA-modulin. Using this cell line, Dr. Baraldi has shown that the activation of GABA receptors increses the Cl^- fluxes and that 1,4-benzodiazepines potentiate the increase in the Cl^- influx elicited by GABA receptor agonists. Additional progress in the study of receptors was obtained in spinal cord when Dr. Gentleman showed the presence of a DA-dependent adenylate cyclase. This finding confirms and extends the proposal made by Dr. Commissiong and his colleagues that a dopaminergic pathway is present in the spinal cord.

Previous work from this laboratory had shown that during the transfer of messages from postsynaptic receptors located in neuronal membranes to the nucleus, the translocation from cytosol to the nucleus of catalytic subunits of protein kinase plays a major role. Dr. Kumakura and Dr. Schwartz have provided evidence that neurotubular protein metabolism must be kept intact in order to obtain an efficient communication from cell membrane to the nucleus. Hence, in the primary cultures of chromaffin cells the induction of tyrosine hydroxylase elicited by 8-Br-cyclic AMP, which was shown in the preceding year to depend on protein kinase translocation, can today be inhibited by incubation with low concentrations of vinblastine or colchicine. Such an action by colchicine was confirmed by Dr. Schwartz in glioma C6 using translocation of protein kinase associated with the induction of phosphodiesterases.

Dr. Revuelta has reported that marijuana selectively inhibits the acetylcholine metabolism in the hippocampal cholinergic system. This drug of abuse, by an unknown mechanism causes a stimulation of GABAergic interneurons present in septal nuclei which regulate transsynaptically septal hippocampal cholinergic neurons. It is possible that this action of marijuana is operative in the psychotomimetic actions of the drug. We are studying the mechanisms in detail because it may help to unveil a specific transmitter mechanism where marijuana could act as a specific agonist or antagonist.

Section on Biochemical Pharmacology (Chief: Norton H. Neff, Ph.D.)

Drs. Gentleman and Commissiong demonstrated the existence in rat of an uncrossed nigro spinal dopaminergic pathway. Three types of studies were performed to evaluate the presence of this pathway: 1) unilateral injection of 6-hydroxydopamine (6-OH-DA) into the substantia nigra which contains the majority of neuronal cell bodies that store dopamine (DA) in brain; 2) unilateral electrical stimulation of the substantia nigra; and 3) bilateral electrolytic lesions of the locus coeruleus which contains the majority of neuronal cell bodies that store norepinephrine (NE) in brain. The DA content of striatum and thoracic spinal cord was significantly reduced ipsilaterally to the substantia nigra lesion. Unilateral electrical stimulation of the substantia nigra also resulted in the accumulation of 3-methoxy, 4-dihydroxyphenylacetic acid (HVA) ipsilaterally to the stimulus. Bilateral lesions of the locus coeruleus had no effect on the concentration of DA in any region of the cord. These results are consistent with the notion that there is an uncrossed dopaminergic nigro spinal pathway.

In another series of studies, Drs. Gentleman and Commissiong found that the DA content of the thoracic spinal cord was highest in the dorsal horn (0.4 nmol/g), lower in the ventral horn (0.1 nmol/g) with the zona intermedia (0.2 nmol/g) between the two. The actions of DA on postsynaptic cells of striatum are mediated by an activation of adenylate cyclase. Drs. Parenti and Gentleman, using a membrane fraction prepared from rat spinal cord, demonstrated that DA activated the adenylate cyclase present in these membranes in a concentration dependent manner, with maximum activation at about 50 μM. One half of the maximum activation occurs between 5-10 μM DA. This activation was blocked by neuroleptic drugs. Moreover, seven days after midthoracic section of the cord, the sensitivity to DA stimulation of the adenylate cyclase located below the section was about 10-fold greater than normal.

In support of the hypothesis that long axon DA neurons of substantia nigra innervate spinal cord, Dr. Gentleman demonstrated that electrical stimulation of the substantia nigra induces a time-dependent rise of the cAMP content in the cervical cord that is blocked by prior treatment with haloperidol. In an attempt to enhance our understanding of neuronal communication of spinal cord, Dr. Kayaalp is providing information on the interaction of cholinergic neurons with other neuronal systems in this tissue. Using the muscarinic receptor ligand ^{3}H-quinuclidinyl benzylate, he has demonstrated that muscarinic receptor binding sites are present in the spinal cord; however, they are more numerous in the lumbarsacral cord than in the cervical and thoracic cords. Moreover, ventral horn contains about twice as many sites as the dorsal horn, with the zona intermedia inbetween the two. White matter contains binding sites for the muscarinic ligand, although considerably less than contained in the gray matter. The number of these sites remain essentially unchanged caudally to a spinal cord transection. In addition, Dr. Kayaalp has identified and characterized muscarinic receptors in rat adrenal medulla. Adrenal cortical tissue contained only a few muscarinic binding sites. Denervation of the adrenal gland failed to change the number of binding sites. To extend our study of spinal cord mechanisms regulating neuron to neuron signaling, Dr. Lackovic has obtained preliminary evidence that substantial quantities of serotonin and DA are associated with the spinal nerve roots, the location of these monoamine stores is now being characterized.

Continuation of the studies on the modulation of sympathetic ganglionic transmission by catecholamines revealed that isoproterenol, a beta-adrenergic receptor agonist, had no effect on the dihydroxyphenylacetic acid (Dopac) content of the celiac ganglion; in contrast, the alpha-adrenergic receptor agonist, phenylephrine, decreased the content of Dopac in this ganglion - this effect was prevented by a pretreatment with phenoxybenzamine. The administration of the DA receptor agonist, apomorphine, had no effect on the content of Dopac, but an injection of the DA antagonist, haloperidol, increased the Dopac content.

Dr. Iuvone, using the retinal model system, continued his studies of the biochemical concomitants to persistent trans-synaptic activation of DA-containing neurons. Using immunotitration methods, he found that the increase in the Vmax of retina tyrosine hydroxylase (TH) elicited by prolonged exposure to light is not associated with an increase in the number of immunoprecipitable TH molecules. These results are consistent with the notion that an increase in the Vmax may reflect an increased specific activity of TH. In retina, this enzyme is located in amacrine neurons which have dendritic processes but no axons. Dr. Iuvone's results together with some reports in the literature suggest that the regulation of TH located in dendrites may be different from the regulation of TH located in nerve terminals. In axons, a persistent increase of impulse traffic increases the number of TH molecules while in dendrites the same stimulus only increases the specific activity of TH.

Continuing our collaboration with the Laboratory of Clinical Psychopharmacology on the characterization of monoamine oxidase from schizophrenics, we are determining whether in psychotics the monoamine oxidase activity in tissues other than platelets is low.

Cholera toxin activates adenylate cyclase nonreversibly; thus, an understanding of the molecular mechanisms of this activation might give a new insight into the regulation of adenylate cyclase, an enzyme of great importance in the cell to cell signaling. Ms. Trepel and Dr. Chuang found that cholera toxin catalyzed the ADP-ribosylation of the pituitary hormones thyrotropin, lutotropin. follicolotropin, human chorionic gonadotropin, corticotropin$_{1-24}$ and of two basic proteins, histone subfraction f_1 and protamine. Casein and phosvitin, which are acidic proteins, failed to act as acceptors in the ADP-ribosylation as catalyzed by choleratoxin. These studies may help to reveal the significance of endogenous ADP ribosyltransferases. One aspect of the physiological significance of these transferases is their participation in the formation of interferons. Ms. Trepel, Dr. Neckers (LCP) and Dr. Chuang found that mouse fibroblast interferon, like cholera toxin, enzymatically transfers ADP-ribose from NAD to protamine. The presence of boiled interferon mock interferons or the incubation at 0°C prevented ADP ribosylation. In contrast to mouse fibroblast interferon, a crude preparation of rabbit kidney interferon failed to catalyze protamine ADP ribosylation. These data indicate that only some forms of interferons can catalyze ADP ribosylation, in this diversity may reside a clue to understand a physiological role of some interferons.

Section on Molecular Pharmacodynamics: (Chief: D. L. Cheney, Ph.D.)

The use of stable isotopes to investigate the dynamics of various neurotransmitters stored in specific neuronal pathways of rat brain has continued to yield valuable insight into the transmitter interactions and its pharmacological significance. Dr. Wood, a guest worker from Canada, has demonstrated that an interruption of the cortico-striatal glutamatergic pathway by decortication results in significant decreases in the turnover rate of acetylcholine and GABA stored in intrinsic striatal neurons. The data supports the hypothesi that the cortical input to the striatum is excitatory and acts to modulate intrinsic striatal cholinergic and GABAergic neurons. Studies involving kainic acid induced lesions of the striatum indicate that most striatal cholinergic interneurons receive a glutamatergic input. Another regulatory mechanism for neuronal interactions in striatum appears to be neurohormonal and probably it is mediated by prolactin. When this pituitary hormone is injected intraventricularly it reduces the rate at which ACh is utilized in striatum and hippocampus but not in other brain areas. Since these effects are nullified by destruction of dopaminergic neurons with 6-OH-DA injected into Areas A_9 and A_{10}, the receptor for prolactin may be located in dopaminergic neurons and through such a mechanism, prolactin may reduce ACh utilization in striatum and hippocampus. Dr. Wood also studied the actions of muscarinic receptor blockers on the dynamics of ACh in central cholinergic neurons of various nuclei of rat brain. He found marked regional variations in the influences of muscarinic receptor blockade on ACh turnover rate and content. In some areas, the turnover rate of ACh is increased (hippocampus and thalamus); in another, it is decreased (cortex) and in still others, remains unchanged (striatum). Also the changes in the ACh levels in different brain areas were not uniform and failed to support the tenet that a decrease or increase in the ACh content is an index of an increase or of a decrease in the ACh turnover, respectively. In addition, these results suggest that in those structures in which muscarinic receptor blockers increase the turnover rate of ACh there may be an inhibitory feedback loop which involves at lease one ACh receptor.

Transmitter interaction studies may be used to trace the site of action of drugs. Delta 9-tetrahydrocannabinol (Delta 9-THC) has been studied quite extensively but we still do not know the site or the mode of action of this drug. Dr. Revuelta has shown that neither endorphinergic nor dopaminergic neurons mediate the selective reduction of turnover rate in hippocampal ACh elicited by the administration of Delta 9-THC or muscimol. Moreover, Delta 9-THC produces a two-fold increase in the turnover of GABA stored in those septal nuclei that participate in the regulation of septal-hippocampal cholinergic neurons. Such results suggest that Delta 9-THC inhibits the ACh turnover rate in the septal-hippocampal pathway by increasing the release of GABA from those septal GABAergic interneurons which trans-synaptically modulate the activity of septal hippocampal cholinergic neurons.

Other cannabinoid analogues (nabilone) and (-)-Delta 8-tetrahydrocannabinol endowed with psychotomimetic action appear to be even more potent than Delta 9-tetrahydrocannabinol in reducing the ACh turnover rate in hippocampus, whereas the (+) isomer of Delta 8-tetrahydrocannabinol which is devoid of psychotomimetic action is without effect on the turnover of hippocampal ACh.

The study of the role of central cholinergic neurons in stress has been especially interesting since standard methods to measure ACh turnover involve a constant rate infusion of deuterated ACh precursor lasting several minutes. This infusion requires restraint of the animals for varying time periods which may act as a stressor and thereby modify the ACh turnover in various brain areas. Drs. Brunello and Tagliamonte inserted chronic cannulas into the jugular vein three days before the turnover experiments in order to infuse the ACh precursors at constant rate in unaesthetized rats. The cannulas emerged through the skin at the back of the neck and were protected by a coiled spring which was long enough to allow the rat to move freely inside the cage. There were no significant differences in the turnover rate of either ACh or GABA measured in various brain areas of restrained or unrestrained rats. Using this procedure of chronic cannula implantation, Drs.

Brunello and Tagliamonte studied the effect of cold stress on the turnover of ACh in various brain areas. They have shown that 1 or 4 hrs of exposure to cold selectively decreases the turnover rate of ACh in the frontal cortex, whereas after 24 hrs of cold exposure the values are normal. The studies of effect of stress on ACh turnover are being extended to cholinergic neurons of adrenal medulla and other peripheral nervous tissues.

Dr. Peralta (guest from Spain) is developing a specific and sensitive gas chromatography-mass fragmentographic method to determine the concentration of enkephalins in neuronal tissue. Using the dipeptidyl aminopeptidase I to cleave enkephalin into dipeptides (Tyr-glyc + glyc-Phe), Dr. Peralta demonstrated with striatal extracts that the adsorption on Biobeads SM-2 of the supernatant and a successive thin layer chromatography of the methanolic eluate are sufficient to exclude the interference from other peptides. Enkephalin can be identified and measured using appropriate internal standards with deuterated amino acids.

Group on High Performance Liquid Chromatography-- Leader: J. L. Meek, Ph.D.

One of the major problems in measuring the neuropeptide content of brain areas has been the development of appropriate assays which are both sensitive and specific. Radioimmunoassays often do not possess the required specificity, because a high degree of molecular heterogeneity is often present in brain polypeptide stores. Drs. Meek and Schmid have obtained better methods to assay neuropeptides using high pressure liquid chromatography, HPLC. This technique has the greatest resolution power of any available technique to physically separate peptides of less than 5,000 MW. Using HPLC, they have made remarkable progress in achieving separations of peptides ranging in size from 5 to 20 amino acids. This methodology is now sufficiently developed that the parameters for separation can be designed directly from a knowledge of the amino acid composition of the peptides. Using this separation technique they are attempting to measure peptides in crude tissue extracts. Although it is possible that HPLC alone might be used for retina peptide measurements, the most specific and sensitive method which can now be envisioned would be to combine the high separation capacity of HPLC with the tremendous specificity and sensitivity of mass spectrometry. Drs. Meek and Peralta are now doing pilot studies to engineer the fusion of these two techniques.

Drs. Schmid and Hong have applied an automated sensitive HPLC method to measure amino acids, to study the effects of kainic acid and electrolytic lesions on various afferents on the hippocampal content of various amino acid transmitters. These studies are giving important information on the wiring of hippocampal pathways containing transmitter amino acids. Moreover, they appear to suggest that thaurine may have a transmitter role in hippocampus.

Section on Neuroendocrinology (Chief: A. Guidotti, M.D.)

The interactions between 1,4-benzodiazepines and GABA receptors were investigated further, both at behavioral and molecular levels. The behavioral studies have proved that in tests designed to reveal the anxiolytic action of 1,4-benzodiazepines, GABA and benzodiazepines are synergists. Dr.

Cananzi (a guest worker from Italy) has shown that intracerebral muscimol mimics the action of 1,4-benzodiazepines to suppress the behavioral depression elicited by conditioned fear. This observation together with previous reports showing that the action of 1,4-benzodiazepines on operant behavior is inhibited by GABA receptor antagonists and potentiated by inhibitors of GABA degradation, strongly indicates that also the anxiolytic action of 1,4-benzodiazepines is mediated by the facilitation of GABA transmission.

During the past year, evidence was obtained suggesting that 1,4-benzodiazepines and GABA interact at the postsynaptic GABA receptor. Dr. Toffano Dr. Baraldi, Dr. Schwartz, and Dr. Suria (George Washington University) and Mr. Konkel have shown that in vitro the stimulation of the benzodiazepine receptors located on brain membranes or on membranes from a neuroblastoma cell line (NB_{2A}) uncovers a population of GABA receptors endowed with high affinity recognition sites for GABA. Conversely, when the GABA receptors of brain membranes are stimulated, the affinity of the specific recognition sites for 1,4-benzodiazepine receptors is increased. This reciprocal interaction between 1,4-benzodiazepines and GABA receptors appears to be mediated by an endogenous regulatory protein (GABA-modulin) which allosterically decreases the number of high affinity recognition sites of GABA receptors. Since the rank order of potency of the various 1,4-benzodiazepines to block the inhibitory action of GABA-modulin on GABA receptors is related to their capacity to displace the specific binding of radioactive benzodiazepines, the data suggest that, in fact, the interaction between 1,4-benzodiazepines and GABA modulin might play a role in the pharmacological action of 1,4-benzodiazepines. In fact, the displacement of a radioactive benzodiazepine specifically bound to receptors by various 1,4-benzodiazepines correlates with their therapeutic activity. Drs. Toffano and Leon have purified GABA-modulin by about 1500-fold. This protein is thermostable, has a molecular weight of approximately 15,000 and is acidic (I.P. 3.2). Since the work of Drs.Leon,Toffano and Szmiglielski (guest from Poland), has indicated that GABA-modulin is an inhibitor of protein kinases, We have explored whether GABA-modulin controls GABA receptor function by altering the state of membrane protein phosphorylation. Indeed, in membranes deprived of GABA-modulin there is an enhancement of protein phosphorylation which is associated with an increase in GABA binding. These and other data suggest that in brain synaptic junctions GABA-modulin regulates GABAergic transmission by controlling the plasticity of GABA receptors via an inhibition of protein phosphorylation; GABA-modulin could represent a point of convergence for the action of 1,4-benzodiazepines in GABA transmission. Because of this emphasis on protein kinases their ontogenetic development has been characterized: Dr. Bandle has shown that in cerebellum, the protein kinase activity increases after birth, in conjunction with the development of innervation.

Dr. Massotti (a guest worker from FDA) and Mr. Konkel have also obtained evidence that GABA modulin and 1,4-benzodiazepine receptors may undergo changes after acute or chronic doses of 1,4-benzodiazepines or after chronic exposure to electroconvulsive shock. Dr. Massotti has shown that in rats born from mothers treated during pregnancy with 1,4-benzodiazepines there is a sustained decrease in the affinity of the recognition sites of 1,4-benzodiazepine receptors. Using the neuroblastoma cell line containing GABA receptor, Dr. Baraldi has shown that stimulation of GABA receptors causes an increase in the rate of Cl^- influx leading to a higher steady state of Cl^- inside the cell. 1,4-Benzodiazepines failed to act on Cl^- flux but facilitated the action of GABA.

In the past, we have shown that in chromaffin cells of rat adrenal medulla, the sequence of molecular events elicited by a persistent activation of nicotinic receptors includes an increase of the cAMP concentration, an activation of cytosolic cAMP-dependent protein kinase, the translocation of the catalytic subunit of protein kinase from the cytosol to the nuclear fraction, an activation of RNA polymerase II, an increase in protein synthesis and the induction of several proteins including tyrosine hydroxylase. Transection of the splanchnic nerve following the increase of cAMP and the translocation of the catalytic subunit of cytosol protein kinase to the nucleus, fail to prevent the induction of TH. Thus, by this sequence of events, nicotinic receptors control nuclear RNA transcription. To study in more detail whether this sequence of molecular events is essential to elicit TH induction, Dr. Kumakura using primary cultures of adrenal chromaffin cells, has shown that also in primary cultures of adrenal chromaffin cells, the induction of TH elicited by cAMP, is also mediated by a nuclear translocation of cAMP-dependent protein kinase and both the induction of TH and the increase in nuclear phosphorylation are blocked by antimicrotubular drugs.

Recent work from this laboratory has shown that [met^5]-enkephalin and enkephalin-like material is present in the adrenal medulla. This material is located in different anatomical pools: the terminals of splanchnic nerve represent an important storage site of [met^5]-enkephalin. The primary culture of chromaffin cells from bovine adrenal medulla represents an ideal model to study the molecular mechanisms operative in expressing the neuromodulation of nicotinic receptor function by enkephalins. Presumably, these pentapeptides are released from splanchnic nerve terminals together with ACh by nerve impulses. Dr. Kumakura has shown that in primary cultures of chromaffin cells the membrane possesses opiate receptor binding sites with characteristics similar to those of brain and that stimulation of these opiate receptors results in a decrease in the number of alpha-bungarotoxin receptor binding sites and in a marked decrease in the release of catecholamines elicited by maximally active doses of nicotine. The role of the opiate receptors was investigated in the nigro striatal dopaminergic pathway and in the hypothalamus. Drs. Moroni, Kumakura and Gale (PRAT Fellow) have shown that opiate receptors control the function of the nigro striatal dopaminergic neurons by interacting with terminals of GABA or substance P axons of substantia nigra. Dr. Grandison has shown that the release of prolactin is under a tonic control of opiate receptors. In fact, intrahypothalamic injections of naltrexone reduced, while that of opiate agonists increased the prolactin release. In addition, the evidence provided by Dr. Grandison suggests that opiate receptors may be located on cells containing prolactin releasing factors since median eminence DA, serotonin, or GABA neurons do not appear to be operative in mediating the effect of opiate receptor stimulation.

The hypothalamus is a brain area where the regulation of DA neurons play a dominant role in controlling hormone release from pituitary. Dr. Grandison has re-examined the role of factors other than DA in the control of prolactin release. Operating with a model in which prolactin release was induced by injecting haloperidol, Dr. Grandison has demonstrated that muscimol, is a potent inhibitor of haloperidol-induced prolactin release. Dr. Grandison and Dr. Baraldi have shown that the action of muscimol on prolactin release occurred on the pituitary and not at the hypothalamic level. Moreover, the inhibition of prolactin release was not due to products of muscimol degradation. With these studies, evidence has been provided for the first time that GABA receptors located in pituitary function in the control of prolactin release, this control operates independently from the DA regulation of prolactin release. From a pharmacological and therapeutic point of view, these results predict that peripheral GABAmimetics may represent a relevant approach to control the prolactin releasing action of the neuroleptics without altering their central effects.

Drs. Lucchelli (guest worker from Italy) and Vargas (guest worker from Mexico) have shown that after chronic treatment with antischizophrenic drugs, the striatal content of calmodulin (a calcium binding protein present in postsynaptic membranes) increases in the striatum but not in the N.accumbens and in other brain areas. Since calmodulin facilitates the stimulation of DA receptors by the agonist, it was tempting to see whether calmodulin could be operative in determining the facilitation of dopaminergic transmission in schizophrenia. However, Dr. Vargas, failed to find a decrease of calmodulin content in the caudate and N. accumbens of schizophrenic patients.

Section on Molecular Neurobiology (Acting Chief: E. Costa, M.D.)

A method to evaluate the action of drugs on peptidergic neurons requires the measurement of the drug induced change in the rate of turnover of these putative transmitters. A measurement of this turnover rate can be obtained by estimating the flux of labeled amino acids into the peptide pools at various times after the infusion of the labeled amino acid. However, the molecular heterogeneity of the neuropeptide stores and the presence of precursors hamper these studies. For instance, in the case of enkephalin, the presence of a pro-enkephalin pool is documented only indirectly; therefore, the measurement of this pool is at best difficult; hence kinetic studies involving the labelling of the pro-enkephalin pool of various brain areas are virtually impossible. Dr. Hong has obtained a rough indication of enkephalin turnover by blocking polypeptide synthesis with cycloheximide and measuring the decrease in striatal enkephalin content at various times thereafter. When similar experiments were carried out after chronic haloperidol treatment, it was suggested that as a part of the changes elicited by the persistent blockade of dopaminergic receptors, there was an increase in the synthesis of striatal enkephalin. Since this increase is associted with an elevation of the pro-enkephalin pool size it is suggested that the blockade of DA receptors causes a trans-synaptic induction of an enzyme that plays a major role in the synthesis of pro-enkephalin. Whether this change plays a role in the therapeutic action of haloperidol remains to be elucidated.

Dr. Hong has studied the effect of recurrent convulsions on the content of enkephalin in hippocampus, his findings suggest that enkephalin turnover increases during convulsions. This line of investigation implicates these peptidergic neurons in the convulsive state. Heterogeneity of polypeptide pools immunochemically related to enkephalins was investigated by Dr.

Di Giulio in various peripheral tissues. These studies revealed the existence of a new polypeptide system in sympathetic ganglia, adrenal medulla, heart, salivary gland and arteries. This polypeptide cross reacts with enkephalin antibodies and inhibits electrically evoked contractions of the guinea pig ileum. Ms Majane found that these effects are naloxone resistant. The new polypeptide is inactivated by incubation with pronase and chemotrypsin but not by amino or carboxypeptidase. The lack of a specific receptor antagonist is a major drawback in the biological characterization of this new peptide. Dr. Yang has provided indirect evidence suggesting that adrenal medulla may secrete this peptide into the blood stream. In collaboration with Dr. Hanbauer (NHLBI), Dr. Hexum (guest worker from the University of Nebraska), Dr. Yang is studying the content of this enkephalin-like material in the venous blood coming from medulla. Dr. Di Giulio has reported that the content of these enkephalin-like peptides is decreased in sympathetic ganglia and adrenal medulla of spontaneously hypertensive rats. This defect is present in young rats before the onset of hypertension. This evidence and preliminary pharmacological studies suggest that this new peptide may be operative in blood pressure regulation. Thus, ongoing studies not only implicate that brain enkephalin operates in releaving psychoses elicited by the neuroleptics but also suggest that enkephalin-like peptides regulate blood pressure. These implications have prompted Dr. Stine to investigate the effects of inhibitors of polypeptide metabolism on analgesia and metabolism of intraventricularly injected [met^5]-enkephalin. This study indicates that dipeptidylcarboxypeptidase is not involved whereas carboxypeptidase A may be operative in the metabolism of [met^5]-and [leu^5]-enkephalins.

Beta-endorphin is present in anterior and intermediate lobe of the pituitary and in the hypothalamus. Dr. Fratta has shown that the regulation of hypothalamic beta-endorphin differs from that of pituitary beta-endorphin. High doses of dexamethazone block the formation of pituitary beta-endorphin but not that of the hypothalamic hormone. Therefore, after a load of dexamethazone the beta endorphin content of the pituitary is reduced, while that of the hypothalamus is normal.

Dr. Govoni has initiated the study of a novel neuropeptide, bombensine, which is present in the hypothalamus and myenteric plexus. The molecular heterogeneity of the tissue stores of this polypeptide was studied with emphasis on its use as a tool to understand how bombensine content is regulated during physiological utilization. The substance P content was measured in sympathetic ganglia and the precise location of this ganglionic store was investigated by Drs. Schwartz and Robinson by lesion experiments. Substance P appears to be located in small cells that are intrinsic to the ganglion. Substance P is also stored in salivary gland, this store appears to be functionally or anatomically related to the corda tympani. Dr. Schwartz has shown that nerve growth factor promotes the accumulation of substance P in sensory ganglia isolated from 9 day old chick embryos.

Subsensitivity to isoproterenol can be induced in beta-adrenergic receptors of frog erythrocytes by incubating these cells with beta-adrenergic agonists. Dr. Chuang investigated the mechanisms of this subsensitivity and found that internalization of the beta adrenergic receptor may be involved. This phenomenon is temperature dependent and requires ATP. Using these properties the kinetic profile and the turnover of beta-adrenergic receptors will be studied by Drs. Kinnier and Chuang.

Dr. V. Chikvaidze (guest worker from Georgia, USSR), Dr. R. Hollenbeck Dr. Kinnier and Dr. Chuang have studied the ontogenesis of protein kinase. They found that Ca^{2+} dependent membrane bound protein kinase increases during the ontogenesis of synapses, whereas the cAMP dependent soluble enzyme fails to change during ontogenesis. This brings into focus the synaptic role of Ca^{2+} dependent protein kinase.

Dr. Schwartz has shown that incubation of C6 glioma cells with isoproterenol elicits an increase in cyclic AMP content, followed by an activation of cyclic AMP dependent protein kinase. In addition she showed that the cytoplasma of these cells contain Type II protein kinase but only a small amount of cyclic AMP independent protein kinase. Following persistent activation of cyclic AMP dependent protein kinase elicited by isoproterenol the catalytic subunits of this enzyme redistribute and concentrate in the particulate fractions. A maximal increase in nuclear protein kinase activity occurs 45-60 minutes following isoproterenol. The addition of cyclic AMP or of Ca^{2+} with or without the specific ionophore, A 23187, fails to increase the nuclear kinase activity in normal and isoproterenol treated cells. Preincubation of these cells with vinblastine blocks the increase of nuclear protein kinase activity due to isoproterenol. If the incubation with vinblastine follows the protein kinase translocation elicited by isoproterenol it fails to reduce the increase in nuclear protein kinase activity elicited by isoproterenol. Since other reports have suggested that translocation of protein kinase is an artifact of homogenization, Dr. Schwartz has examined this function closely and concluded that translocation represents a functional and physiological response to beta adrenergic receptor stimulation. Moreover, her results show that translocation is a system to bring about communication betwee cell membranes and nuclei. This conclusion is supported by her finding that the induction of phosphodiesterase elicited by protracted stimulation of beta adrenergic receptors requires nuclear translocation of protein kinase subunits whereas the increase in beta nerve growth factor content elicited by isoproterenol is not dependent on such translocation.

Annual Report of the Research Services Branch

National Institute of Mental Health

National Institute of Neurological and Communicative Disorders and Stroke

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The Research Services Branch (RSB) provides broad technical support for the Intramural Research Programs of NIMH and NINCDS through (1) research and development in advanced biomedical instrumentation techniques and systems; (2) evaluation, specification and management of computer systems; (3) direction of a program of laboratory animal medicine and care (NIMH only); (4) provision of other technical services in support of the research program.

The Branch is comprised of two sections:

Section on Instrumentation and Computers provides technical support for investigators by (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation and systems not commercially available; (3) designing, specifying and managing laboratory computer systems for data acquisition and processing.

Section on Laboratory Animal Medicine and Care provides professional advice and assistance to intramural scientists on all aspects of animal health, medical care, and testing and surgical protocols; manages a central primate holding facility; and conducts a program of laboratory animal care.

Additional services provided by the Branch include consultation on measurement techniques, signal processing, noise and electro-magnetic interference in data measurement systems, and equipment purchases. Several formal and informal courses for investigators are taught by Branch personnel; topics include electrical circuit theory, operational amplifier applications, digital logic design, and computer applications.

Due to manpower limitations and economic considerations, the Branch is unable to provide the following services: repair of commercial instruments, duplication of off-the-shelf commercially available equipment, and fabrication of non-instrument items (shelves, bookcases, etc.).

When an investigator requires the services of the Branch, he first meets with the Branch Chief and other personnel as needed to discuss his requirements. On the basis of this meeting, a decision is made as to whether RSB will take on the project. If a commercially produced instrument will satisfy the investigator's requirements, he is advised to purchase it. If custom instrumentation is needed, RSB will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Branch (BEIB).

When the Branch Chief or the Assistant to the Chief agree to accept a project, the investigator submits a standard work request form (available fro RSB), signed by his Lab Chief. This form will state the nature of the instrument or service requested, and will contain as many details and specifications as the investigator can provide.

The project is then assigned to an engineer, who will confer with the investigator to formulate a set of engineering specifications and a timetable and cost estimate for the project. The RSB does not charge for services, but the investigator will be billed for the cost of the components used. Upon delivery of the completed instrument, a memo is sent to the investigator listing the component costs and asking permission to have the Administrative Officer transfer funds from his CAN to the Branch's CAN.

SECTION ON INSTRUMENTATION AND COMPUTERS

INSTRUMENTATION

The following are selected instrumentation projects undertaken during the past year. These are chosen from a total of 281 projects, and are representative of the types of electronic instruments and systems developed by the Branch:

(1) Patient Activity Monitoring System. The Branch has continued to develop a system for monitoring the activity patterns of ambulatory subjects. The development this year was divided into two major areas. First, the continued production of the standard activity monitor, following its third redesign last year, was paced to meet the increased need. Twenty-five new monitors were produced; about half were used to phase-out older monitors with reliability problems and the other half went to expanded or new research programs. The advantages of the newer design (better calibration consistency and longer battery life, using an off-the-shelf battery) are sufficient for the Branch to plan to gradually replace all the older models. Eighty monitors are now distributed among twelve research groups, with approximately 65-70 monitors in active use at any given time.

The second major effort this year was, and continues to be, the development of a more compact monitor (approximately two-thirds the present volume) with a four-fold increase in memory capacity. To accomplish this, a contract was let for the production of miniaturized electronic circuits using thick-film hybrid technology. The first samples of these hybrid circuits will be delivered in the near future, and will undergo checkout using electronic instrumentation developed by the Branch. Once correct function is verified, subsequent deliveries should follow quickly so that production of the complete "hybrid" monitors can progress rapidly. With the capacity for 10 days of continuous recording at 15 minute resolution, the majority of the expected 50 monitors produced from this initial contract are aimed for long-term studies of manic-depressive outpatients.

A new calibration device with improved stability and repeatability of movement has been developed for quantitative characterization of each monitor's sensitivity to simulated patient movement. Up to eight monitors can be attached to a circular platter which is then rotated through + 10 degrees at varying magnitudes of angular acceleration. The platter is driven by a brushless DC torque motor arranged in a closed-loop position control system. The motor commands are under programmed logical control so that acceleration sequences can be accurately repeated, and coordinated with the internal timing of the activity monitors. This device will also see general use as a calibrator for other devices employing accelerometers, such as the tri-axial accelerometer of the neurological function testing station, described below.

(2) Large Screen Display System. A large screen CRT display system has been developed to present complex spatial and temporal visual patterns to monkeys during signle unit recording. In addition to the large format (16" x 12"), the display has high resolution (1024 raster scan lines/frame) with no detectable flicker (120 frames/second). Under control of a PDP LSI-11 microprocessor, the display circuitry can (1) generate arbitrary spatial frequency patterns, either fixed or drifting; (2) instantaneously rotate and/or change position of the visual pattern; (3) limit the area of the pattern without changing intensity; and (4) change from a light-on-dark visual target to a dark-on-light target. These capabilities allow a much greater range of visual experiments than previously possible.

(3) Control Electronics For Tissue Freezing Apparatus. An electromechanical apparatus for very rapid freezing of tissue samples is being refined by the Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS, with the assistance of RSB. Successful and safe operation of this device depends on activation of gas valves, solenoids, and an electromagnet in a precisely timed sequence. An electronic timer-controller has been developed to insure that this sequence is correctly generated with high repeatability over many experimental runs. This automation has greatly reduced equipment and operator error, which in turn, has reduced repeat experiments and wasted time.

(4) Dual Discriminator For Post-Synaptic Potentials. This instrument incorporates a standard RSB window discriminator for pre-synaptic spike detection and a second modified discriminator for preprocessing post-synaptic potentials (PSP). In addition to generating a multiplexed signal for the investigator's display scope, the PSP discriminator provides sampling time and amplitude limit information to a PDP-11 minicomputer which then generates a variety of on-line histogram displays.

(5) Neurological Function Testing Station. A neurological function testing station has been developed to measure the various parameters associated with Parkinson's disease. Three separate tests will be administered by the equipment to obtain data relating to the three major symptoms of the disease: tremor, akinesia, and rigidity. The hardware developed for the system interfaces to a small laboratory computer, the Plessey LSI-11/03. The computer controls the experiment, records and analyzes the data.

The electronics developed for this system cover three areas: measurement of tremor, gait, and reaction and movement time. Tremor is recorded with a tri-axial accelerometer with circuitry incorporated to allow a measure of tremor frequency and amplitude. The gait of the subject is measured with a 22 foot long treadway with pressure sensitive switches every 4 inches, enabling the step length and elapsed time to be recorded. The last test performed is for reaction and movement time. This test is done by means of two capacitive-sensitive touch pads. The pads are designed with three circular "zones" that allow a measure of accuracy during the test.

Although designed initially for use in Parkinson's disease studies, the system will eventually be used in other studies involving recording of similar neurological parameters.

(6) Hamster Activity Recording System. An expanded system for measuring and recording the activity of 32 animal exercise wheels was developed. A photoelectric detector on each wheel and interface circuitry allows an LSI-11 computer to monitor each of the 32 input lines and record each rotation. The total number of counts for each cage is recorded every 15 minutes on a cartridge tape system, and displayed on a screen. Periodically the data recorded on the cartridge recorder can be transferred to a larger computer system for plotting and further analysis.

(7) Muscle Rigidity Measurement. A system was developed to measure resistance to passive stretch of a rat's hindfoot. Such resistive force is a measure of muscle rigidity, which is used when studying the pathophysiological mechanisms underlying akinesia and Parkinsonian rigidity. Resistance is induced by extending a metal beam against the lower part of a rat's hindfoot while the leg is held fixed. The foot is dorsiflexed and held for a given period and then the beam is retracted. A second time period elapses before the beam is again extended. Stretch resistance is measured by determining the strain in the metal beam used to dorsiflex the animal's limb. Resistive force can accurately be resolved to 1 gram-force over a 500 gram-force range using semiconductor strain gages and an integrated circuit strain gage conditioner.

Beam position is controlled by a digital linear stepping motor under command of a microprocessor which takes inputs from thumbwheel switches; 0.1 to 9.9 mm stepping distance; 1-99 sec. extension time; 10-99 sec. waiting time; and 1-99 trials per test run. An internally stored program calls the input values and operates the stepping motor as prescribed. Such microprocessor control offers accurate and reliable beam positioning and test run timing.

(8) Rat Sleep Scorer. Efforts are continuing to develop a device to categorize rat sleep state by analysis of bifrontal and frontal-occipital electrocortical activity and electromyographic activity. The desired device would convert such activities in real-time and provide a hard-copy record of total time in each sleep state, as well as a chronological record of 10-sec. epochs. It is hoped to improve an achieved accuracy rate of approximately 80% by better understanding the current human-scorer methods and also by analysis of spectral power of the brain waves.

(9) Strain Gage System. A strain gage system has been developed to measure finger pressure on the handle of a torque motor position control system used for research on the mechanisms which produce tremor of Parkinson's disease. The output is a voltage proportional to the force applied

by the primate's fingers; accurate and reliable resolution to 0.1 gram-force over a 250 gram-force range has been achieved. The circuitry was implemented with a new, integrated circuit strain gage conditioner. It consists of a high performance instrumentation amplifier with 120 db common-mode rejection and adjustable gain to 1000 V/V; a three-pole, low-pass filter having 60 db/decade roll-off and an adjustable corner frequency; and an adjustable excitation voltage stage.

(10) Rat Rotation Monitor. This previously reported device transduces and counts the clockwise/counterclockwise rotations of a rat. It is used in pharmacology studies. During the past year a modified design was implemented to expand memory storage of total counts per interval to service eight animals for 32 intervals. Available time intervals range from 5 to 60 min. in 5 min. increments. The device has been used on a more widespread basis in NIMH and NINCDS, and details of the device were published in the literature.

(11) Rat Activity Monitoring Cage. The activity cage developed by the Branch has been modified to decrease inter-cage variations in the measurement of activity. The cage electronically senses the location and rearing movements of an unrestricted rat located within the cage. This is accomplished through the measurement of capacitance changes induced by the rat's positioning on four large plates placed one on each of the four sides of the cage. The modifications included upgrading some electronic components to newly developed devices with better specifications. Other, more sensitive, methods of capacitance measurement are being studied to further improve the performance of the cages.

(12) Electromagnetic Muscle Stretch Device. A technique for stretching individual muscles in intact behaving animals via chronic intramuscular implantation of a magnetically permeable slug and use of an external electromagnet to apply force to the slug, has been developed for use in the study of the role of muscle sensory receptors in skilled motor activity. The slug is surgically implanted in the musculotendinous junction in the forearm. The arm is placed inside a solenoidally wound electromagnet; current through the coil produces a magnetic field which exerts a force on the slug, and thus on the muscle. The placement of the slug in relation to the coil and the coil current determine the direction, amplitude, and frequency of the force on the muscle, and thus determine which receptors are stimulated. Developments this year included a comprehensive analysis of the theory and practical considerations of the interaction between coil and slug, to be published in the engineering literature.

(13) Torque Motor Position Control System. A system was developed for research on the mechanisms which produce the tremor of Parkinson's disease. The system, consisting of brushless DC torque motor, handle, housing, power amplifier, and control electronics, was designed to give controlled mechanical stimuli to the wrist joint. The control system operates in an open loop torque mode or a closed loop position control mode. Switching is done electronically under experiment control. Two systems have been developed: one for human subjects, and a smaller version for use by monkeys.

(14) Remote Audio Cassette Data Entry Logger. This project configured a DEC VT40 computer terminal and an analog cassette recorder as a stand-alone data entry point for observational comments regarding monkey behavior. Facilities were also provided for cassette play-back at a later time at a DEC PDP-12 for correlation of observational comments with other physiological records recorded on another media. The VT50 TTY serial output is fed back to the TTY serial input in order to provide the operator a display of the text going onto tape. Then a portion of the TTY serial output was tapped and conditioned for analog recording. At play-back the analog data is reformatted into a serial data stream and recovered at a PDP-12 A/D input channel.

COMPUTERS

The Research Services Branch continues to support the use of the computer as a laboratory instrument. Small computers are used in the individual laboratories for on-line, real-time interaction, process control and data acquisition. RSB maintains support computers in Buildings 10 and 36. These systems provide means for program preparation, bulk storage, printing and plotting capabilities, and minor mathematical and statistical processing. Experimental data may be transmitted from the laboratory computers, via these systems, to the DCRT facilities for further processing. The support computers also serve to develop prototype systems and to test the feasibility of the use of a computer in specific laboratory applications. The latter capability allows an investigator, once he determines that the computer will do the job, to purchase an efficient system at minimal cost.

The system for automated analysis of autoradiographs that was developed for the Laboratory of Cerebral Metabolism (LCM), proved so successful that the facility was soon overloaded. Preliminary studies demonstrated that the usefulness of an interactive system for the graphic analysis of photographic images extends far beyond autoradiographs. Examples are the study of neural processes, results from a PET scanner and two-dimensional electrophoretic gels. A systems study was conducted to develop an improved configuration for such analysis. Two systems were purchased: an additional system for the LCM to increase their production; and a system to be maintained by the Research Services Branch to provide image scanning services to NIMH and NINCDS. This system will be used to continue developmental studies aimed at extending the usefulness of such systems.

The Branch provides software support for the individual investigators. A library of procedures has been developed that are tailored to the needs of the Intramural Program. Individual training is available for beginning investigators. Computer specialists are available for consultation for all areas of computer use, programming, interfacing, real-time applications, time series analysis, data presentation, systems configuration and computer procurement. Although RSB does not provide an applications programming service, systems have been developed in collaboration with individual laboratories. Examples are included in the list of computer related projects.

An increasing amount of time is devoted to program maintenance. Computer programs, especially in research related services, are not static and often require development, improvement or modifications. In addition to the software library and research related projects developed by the Branch, much work is caused by the turnover of scientific and support personnel. Many systems developed by these persons prove useful to the laboratory. After they leave, maintenance of such systems often falls to the personnel of the Branch. The Branch has investigated the use of structured programming techniques and programming languages in an attempt to define software standards. PASCAL was chosen as a standard language. As the problem of program maintenance continues to grow, use of these techniques will be necessary to enable the Branch to furnish this service without an increase in personnel.

One of the major functions of the Branch is to provide systems studies for the use of the computer as a laboratory instrument. The concept of the micro-computer as an integral part of laboratory instrumentation for process control, data logging, timing and coordination of instruments has proven to be both cost effective and efficient for laboratory applications. Mini- and micro-computer based systems provide a wide spectrum of tools for research ranging from lower cost data loggers to sophisticated systems which interact with the on-going experiment. These systems have resulted in a further integration of the engineering and computer functions of the Branch and have enabled the Branch to offer a wide range of computer related services. Projects such as the Neurological Function Testing Station are the result of close coordination between the scientist, the electronic engineers, and the computer scientist and will provide a service not feasible a few years ago.

Examples of computer-related projects include:

(1) Membrane Activity of Neurosecretory Cells. This system was developed in collaboration with the Laboratory of Neurophysiology (NINCDS) to study the conductance of cell membranes that often exhibit bursting activity during clamping. The system is being upgraded to record and monitor the tissue culture neurons on-line and to allow the iontophoretic or pressure injection of neuroactive substances onto the external surfaces of the cells. The purpose is to discover the nature of the action of such substances on the properties of the cell membrane.

(2) Cell Culture Analysis. This system is designed to provide an on-line analysis of tissue culture neurons. The first phase, to study the excitatory or inhibitory post-synaptic potentials of these cells, has been completed. A unique feature of this system is the on-line control of artifacts introduced by the measurement system and the properties of tissues in culture and to control the threshold levels and amplification level as the experiment is in progress. Visual displays of amplitude, integral and latency are available, as well as averaged evoked response. In addition, on-line monitoring of post-synaptic potentials elicited by stimuli presented in pairs or in trains of pulses are available. The system also studies spontaneously occurring miniature potentials. This system will be extended to allow analysis of the cells by other techniques such as voltage clamping and the iontophoretic injection of neuroactive substances on the surface of the cell.

(3) Rat Activity Monitoring System. The system designed to measure gross movements and rearing activity of white rats was extended to differentiate between two levels of gross movements and to monitor the amount of time the animal spends in each quadrant of the cage.

(4) Membrane Channel Analysis. Acetylcholine molecules outside neural membranes open and close channels allowing the passage of Na+ and K+ ions. Current fluctuations resulting from single channels opening and closing have a high signal-to-noise ratio. This program catalogues and identifies the duration and amplitude of channel activity.

(5) Cytofluorographic Analysis of Single Cells. The system designed for cytofluorographic cell sorting by the Computer Systems Laboratory, DCRT, is being modified for the cytofluorographic analysis of cells in immunological studies conducted by the Immunochemistry and Clinical Investigations Section, Infectious Diseases Branch, NINCDS.

(6) Data Acquisition System For Rat Vocalization. A data acquisition and display program has been written for a PDP-11/04 to sample and store the video signal of a Tektronix 5L4N spectrum analyzer. The audio transducer of this device is placed within the 'rat universe' of the Laboratory of Brain Evolution and Behavior, NIMH. The program selectively stores only those vocalizations within a frequency range specified by the experimenter, and records the time of the vocalizations. The results can be displayed on a video terminal or line printer. Further analysis of this data is yet to be done.

(7) Digital Filter Design and Applications. A package of FORTRAN programs has been implemented on the PDP-11/40 which is used to design digital filters for use in off-line signal processing applications. Although the original algorithms were obtained from the open literature, this system implements the algorithms in an interactive way and contains support programs which display and use the filters in a convenient manner. The results of this package have been used in various other programming systems.

SECTION ON LABORATORY ANIMAL MEDICINE AND CARE

The Section on Laboratory Animal Medicine and Care is staffed by a veterinarian, laboratory animal technicians and animal caretakers who provide daily care and maintenance of laboratory research animals. Services provided by the Section include:

(1) Management of the Central Primate Facility. The Central Primate Facility has a capacity for approximately forty primates. At the present, three investigators have research projects in progress. Support is provided through assistance in preparing surgical models, insertion of catheters and maintaining animals on special diets and feeding routines. Eighty-five anesthetic and/or surgical procedures were supported this past year. The Section has also developed the capability for bacteriological cultering and identification.

(2) Tuberculosis Testing Program. All primates, including those located at St. Elizabeth's Hospital are tuberculosis tested every three months.

(3) Treatment of sick and post-operative animals.

(4) Assistance in animal procurement when needed.

(5) Providing day to day care and maintenance of research animals. Care and maintenance of research animals are provided seven days a week in all areas. The supervisor of the Section provides day to day contact with personnel in all work areas and coordinates weekend and holiday schedules. Night coverage of the primate nursery continues to work very smoothly with two part-time persons covering on alternating nights. Also, these two persons administer night medications to animals on treatment.

(6) The Upward Mobility Training Program was reinitiated this year and another person has been selected to start training in July. The goal of this program is to select personnel for further training who have become proficient in their job but lack a career ladder to learn new skills and techniques for further advancement. The training period lasts for eight months, after which the trainee returns to his former duties with qualifications for advancement to the biological technician (animal) field.

(7) Animal Facilities. Upgrading of the animal holding facilities continues. New rodent, rabbit and primate cages for the animal rooms in Building 36 have been received and are in use. Rat and hamster cages for the Poolesville facilities have also been received. Laminar flow units for the long-term holding of rodents were ordered and received for use at St. Elizabeth's Hospital. A new cage washer, purchased by NINCDS, was recently installed in Building 9, replacing one which was twenty-five years old.

Although the animal holding facilities at St. Elizabeth's Hospital and Poolesville are not part of the Section due to logistic difficulties, the veterinarian is responsible for the quality of animal care delivered. Consultant visits are made periodically to assist in care and maintenance of research animals and to take care of health problems that may exist.

MAY BE VIOLATED UP TO 2 OR ... LETTERS.

ENGINEERING, COMPUTER AND FABRICATION SERVICES

This table shows the distribution of the Branch's workload among the various laboratories.

LABORATORY OR BRANCH	HOURS	PERCENT
Experimental Therapeutics, NINCDS	3510	11.07
Neurophysiology, NIMH	3326	10.49
Neuropharmacology, NIMH	2963	9.34
Clinical Psychobiology, NIMH	2874	9.06
Clinical Science, NIMH	2856	9.00
Developmental Neurobiology, NICHD	2776	8.75
Neurophysiology, NINCDS	1668	5.26
Neuropathology and Neuroanatomical Sciences, NINCDS	1474	4.64
Neurobiology, NIMH	1362	4.30
Brain Evolution and Behavior, NIMH	1305	4.29
Biological Psychiatry, NIMH	1175	3.70
Biophysics, NINCDS	1043	3.28
Neurochemistry, NIMH	1011	3.18
Neuropsychology, NIMH	981	3.09
Cerebral Metabolism, NIMH	642	2.02
Molecular Biology, NINCDS	615	1.93
Neuro-Otolaryngology, NINCDS	546	1.72
General and Comparative Biochemistry, NIMH	490	1.54
Infectious Diseases, NINCDS	372	1.17
Neurochemistry, NINCDS	270	.85
Neuroimmunology, NINCDS	218	.68
Central Nervous System Studies, NINCDS	164	.51
Neural Control, NINCDS	62	.19
NIMH (Total)	18,985	59.89
NINCDS (Total)	9,942	31.36
NICHD (Total)*	2,776	8.75
	31,703	100.00

*NICHD loans the Branch one position, and is thus entitled to 1700 hours of service.

IMPORTANT: (1) SINGLE SPACE, EXCEPT BETWEEN PARAGRAPHS.
(2) USE REASONABLY NEW BLACK TYPEWRITER RIBBON, PREFERABLY CARBON PAPER RIBBON.
(3) "OFFSET REPRODUCTION" RIBBON IS NOT NECESSARY OR DESIRABLE.

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